(48) Although Hayat and Silver (ref 12) calculate $\theta = 17°$, using their procedure and data we obtain $\theta = 12°$ which corresponds to $\phi = 36°$ and $q_{NN}{}^{N}(36°) = 24.4$ G.

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Estimating Microsecond Rotational Correlation Times from Lifetime Broadening of Nitroxide Electron Spin Resonance Spectra Near the Rigid Limit[1a]

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A simple method of estimating rotational correlation times (τ_R) of the order of microseconds using the widths of the outer esr hyperfine extrema is discussed. An earlier method allowed determination of τ_R from the ratio of the outer hyperfine extrema separation at a given τ_R to the rigid limit separation. The present method, however, permits the determination of τ_R even when the separation of the outer hyperfine extrema is experimentally indistinguishable from the rigid limit value. It should be useful for many macromolecules with a rigidly bound nitroxide spin label, since they typically exhibit values of τ_R on the order of microseconds in solution.

There have been recent efforts in developing simplified methods for estimating rotational correlation times from the slow-motional esr spectra of nitroxide free radicals.[2] They are based on measuring the shifts, arising from the motion of the hyperfine extrema from their rigid limit values. In particular one need only measure $S \equiv A_z'/A_z$ where A_z' is one-half the separation of the outer hyperfine extrema and A_z is the rigid limit value for the same quantity.[2] Such methods become very insensitive for motions with rotational correlation times $\tau_R > 1 \times 10^{-7}$ sec where $(1 - S) < 3\%$ and, furthermore, in the region $\tau_R > 10^{-8}$ sec the predicted results are very sensitive to a choice of residual width δ.[2a] There has also been interest in extending the range in which τ_R can be measured to longer times by means of more sophisticated techniques such as saturation studies[3] and modulation-frequency-dependent adiabatic-rapid-passage methods.[4]

We have been able to develop a simple technique, based on measuring the line width variations of the outer extrema of the spectra,[5] that may, in many cases, be useful in extending the range of measurable τ_R's to $\tau_R < 5 \times 10^{-6}$ sec. The basic idea is a very simple one. The residual motional broadening in the near rigid limit may simply be regarded as arising from "uncertainty-in-lifetime broadening" due to the rotational motion carrying the nitroxide radical between different orientations corresponding to substantially different esr frequencies (some corrections must, of course, also be made for broadening due to nuclear spin flips). Then one would expect this width contribution to be given, to a rough approximation, by τ_R^{-1}, the reorientational rate. This is indeed found to be the case, to within a factor of 2 (or ½), over most of the range of interest! Furthermore, the greater sensitivity of the widths (*vs.* the shifts in position) of the extrema is analogous to the well-known case of exchange in magnetic resonance where lines first broaden before they shift.[6] We develop a more accurate method and analysis below.

In the rigid limit the outer hyperfine extrema (for an isotropic distribution of nitroxide spin labels) arise from those nitroxide radicals for which the 2p π orbital of the nitrogen atom is nearly parallel to the applied field direction. One may use standard methods to compute such spectra,[7] but we note from a simple analysis of McConnell, *et al.*,[8,9] that the derivative patterns of the outer hyperfine extrema are reasonably approximated as absorption curves with a shape function characteristic of the inhomogeneous broadening. Experimentally, one finds that the outer hyperfine extrema have line shapes ranging from almost perfect Lorentzian to intermediate between Lorentzian and Gaussian.[7,9] (The low-field side of the high-field line and the high-field side of the low-field line are somewhat distorted by overlap with the central region of the spectrum.) We have found that the average of the half-widths at half-heights (Δ) for the two outer extrema of a rigid limit spectrum is, to a very good approximation, equal to $(\sqrt{3}/2)\delta$, where δ is the peak-to-peak derivative Lorentzian line width used in our simulations of computed rigid limit spectra.[7] (The heights of the hyperfine extrema are measured from the true base line (*cf.* Figure 1).) More precisely we have found that

$$2\Delta_l^{r} = 1.59\,\delta \tag{1a}$$

and

$$2\Delta_h^{r} = 1.81\,\delta \tag{1b}$$

(where subscripts l and h refer to the low- and high-field lines, respectively, and the superscript r refers to the rigid limit value). This result is found to be independent of δ

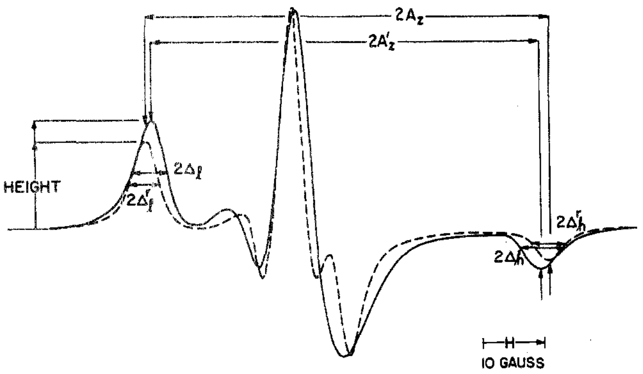

Figure 1. Superposition of computed rigid limit nitroxide spectrum with a computed slow-tumbling spectrum at $\tau_R = 5.0 \times 10^{-8}$ sec demonstrating the measurements required for the parameters $S = A_z'/A_z$, $W_l = \Delta_l/\Delta_l^r$, and $W_h = \Delta_h/\Delta_h^r$. In an actual experiment it is often necessary to estimate the Δ_i^{er} in place of the Δ_i^r as described in the text. The magnetic parameters utilized for this figure are $\delta = 3.0$ G, $g_x = g_y = 2.0075$, $g_z = 2.0027$, $A_x = A_y = 6.0$ G, $A_z = 32.0$ G, $B_0 = 3300$ G.

over the range $1.0 \leq \delta \leq 4.0$ G and virtually independent of A_z over the range $27 \leq A_z \leq 40$ G. It is, of course, essentially independent of variations in the other nitroxide rigid limit parameters. It is a result obtained for the assumption of Lorentzian inhomogeneous broadening.

In the slow-motional region, near the rigid limit, the line width Δ, for Lorentzian line shapes, can be decomposed into two contributions.[10] (1) the Lorentzian inhomogeneous component given by eq 1a and 1b and (2) the excess motional width (of order of magnitude τ_R^{-1}). (It is convenient to think in terms of this decomposition even though it is not necessary for the method below.) A useful dimensionless parameter for describing these spectra is then

$$W_i \equiv \Delta_i/\Delta_i^r \qquad (2)$$

or

$$W - 1 = (\Delta_i - \Delta_i^r)/\Delta_i^r$$

where i = l, h. We have found that, in general $(W - 1)$ is about an order of magnitude larger than $(1 - S)$ for a particular value of τ_R (*cf.* Figure 2), and furthermore it could be measured to *at least* comparable accuracy (~1% *cf.* Figure 1), but note that the $(W - 1)$ parameter would be quite sensitive to distortion of the true line width by over modulation and/or power saturation. Our results in Figure 2 have been calculated from the rigorous theory of Freed, Bruno, and Polnaszek, as was done in I. We have studied how W_i is affected by changes in (1) the spin parameters, (2) line width, and (3) rotational diffusion model. We have found that W_i, like S, is insensitive to deviations from axial A and g tensors used in I, as well as to variations in $A_\perp$, $g_\parallel$, and $g_\perp$, typical of a nitroxide. However, in contrast to S, which is dependent on the product of $\tau_R A_z$, W_i is virtually independent of A_z over the range $27 \geq A_z \geq 40$ G (we have used $A_z = 32$ G in obtaining the results in Figure 2). However, $W_i - 1$ is found to depend upon δ. Generally, a smaller δ implies a larger $(\Delta_i - \Delta_i^r)$ for a given τ_R. In particular, a $\delta = 1$ G yields values of $(\Delta_i - \Delta_i^r)$ ranging from 1.3-2.5 times greater than those for $\delta = 3$ G. A qualitative explanation of this observation is as follows. The rigid limit extrema of finite width Δ_i^r arise from those nitroxide radicals whose 2p π, N atom orbitals lie within a cone of angle Ω about the applied field direction, and the size of the cone increases rapidly with an increase in the rigid limit δ.[8] If we roughly identify the excess width $(\Delta_i - \Delta_i^r)$ with the rate that radicals reorient out of the cone, then extrema from the larger cones (which result from greater values of δ) will be less broadened, since it takes longer for the radicals to leave the cone.[11] Another observation, *viz.* $(\Delta_h - \Delta_h^r)$ is always significantly larger than $(\Delta_l - \Delta_l^r)$, at a given τ_R is explained in a similar manner. It is known that the high-field resonance for a single-crystal spectrum changes with angle more rapidly than the low-field resonance; thus the range of Ω contained in the observed cone (from a polycrystalline sample) must be smaller for the high-field line.[8] Reorientations out of the high-field cone thus occur at a more rapid rate, and, in general, W_h is a more sensitive function of τ_R than W_l as may be seen from Figure 2.

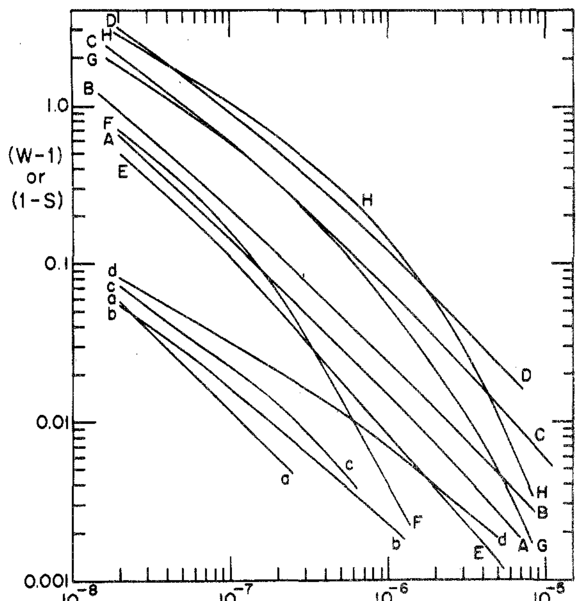

Figure 2. Graph of $(W - 1)$: curves A–H; and $(1 - S)$: curves a–d *vs* τ_R for nitroxide isotropic rotational reorientation from computer simulations of Brownian diffusion and approximate free diffusion. Curves a and b are for approximate free diffusion and derivative widths $\delta = 3.0$ and 1.0 G, respectively, and curves c and d are for Brownian diffusion where $\delta = 3.0$ and 1.0, respectively. Curves a through d may be approximated by $\tau_R = a(1 - S)^b$ to a high degree of accuracy with (b) $a = 5.90 \times 10^{-10}$ sec, $b = -1.24$; (d) $a = 2.95 \times 10^{-10}$, $b = -1.68$. The equations for a and c are given in I. Curves A and B are for approximate free diffusion, $\delta = 3.0$ G, and the low- and high-field extrema, respectively, and curves C and D are the same as A and B, respectively, except δ equals 1.0 G. Curves E and F are for Brownian diffusion, $\delta = 3.0$ G, and the low- and high-field extrema, respectively, and curves G and H are the same as E and F, respectively, except $\delta = 1.0$ G. See also Table I.

TABLE I: Parameters for Relating τ_R to $(W - 1)$[a]

Curve	$a \times 10^8$, sec	b	Max deviation, %[b]
A	1.31	1.033	3
B	1.94	1.062	6
C	5.16	1.076	18
D	8.33	1.125	18
E	1.16	0.943	5
F	2.07	0.778	18
G	5.28	0.999	30
H	10.11	1.014	55

[a] Table is based on approximate fit of Figure 2 data to eq 3 for $(W - 1) > 0.01$. [b] Based on comparing values in Figure 2 with eq 3.

The choice of a proper δ must now be considered. Near the rigid limit the appropriate range of δ for these calculations and those of I can be deduced from the Δ_i^r. The narrowest rigid limit δ found in our laboratory is 1.5 G which corresponds to $2\ \Delta_l^r = 2.4$ G. Hubbell and McConnell[9] report values of 4.6 and 5.5 G for $2\Delta_l^r$ for pseudoaxial "rigid limit" spectra, corresponding to δ of 2.9 and 3.5 G, respectively. The rigid limit spectrum of the *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5α-androstan-3-one appears to have a low-field full-width at a half-height of about 5.0 G, which corresponds to a δ of 3.1 G.[8] (Note that the motional broadening can easily double the widths of the outer extrema when the separation of the hyperfine extrema is essentially unchanged from the rigid limit value (*i.e.*, $S > 0.95$).)

Thus we observe that very near the rigid limit, where δ may be determined from the rigid limit extrema widths, two independent determinations of τ_R can be made from Figure 2.

The major contributions to the Δ_i^r are electron nuclear hyperfine interaction between the electron and the protons of the spin label and host. Heterogeneity of the environment also contributes to the rigid limit line width, because the nitroxide magnetic parameters depend on the details of the environment.[12] Unfortunately, these interactions will be quickly averaged with the onset of molecular motion, resulting in a decrease in the appropriate δ. When this is the case, it becomes necessary to estimate a δ, such that the rotational correlation times obtained from the plots of W_i *vs.* τ_R for both the low- and high-field extrema are equal within experimental error. For this process we may define effective inhomogeneous widths Δ_i^{er} which obey eq 1 and which generally obey $\Delta_i^{er} \leq \Delta_i^r$. Then we may rewrite eq 2 as

$$W_i \equiv \Delta_i/\Delta_i^{er} \tag{2′}$$

This method then permits an accurate determination of both τ_R and δ.

As we have noted, the uncertainty in δ can result in serious errors when τ_R's $> 3 \times 10^{-8}$ sec are determined from S. Once δ has been determined from the W_i, another estimate of τ_R may (when feasible) be obtained from S using either the analytical formulas of this paper and of I, or by linear interpolation of the graphs. This serves as a check on the results from the W_i. In other words τ_R and δ may often be obtained as a function of three experimental parameters, S, and the W_i.

The model-dependent studies shown in Figure 2 were performed for (1) Brownian diffusion where $\tau_R = (6R)^{-1}$ with R the rotational diffusion coefficient and (2) approximate free diffusion, in which $\tau_R = (6B_2R)^{-1}$ with $B_2^{-1} = \sqrt{7}$ yielding results equivalent to a moderate jump model with $|\langle\epsilon^2\rangle_{av}|^{1/2} \approx 50°$.[7] These definitions are chosen so they yield model-independent results in the motional narrowing region for equal values of τ_R.[7] The free diffusion model results in more nearly linear dependence (in a log-log plot) of $W - 1$ *vs.* τ_R in Figure 2 than the Brownian motion model. We have fit the plots in Figure 2 to the form

$$\tau_R = a(W - 1)^{-b} \tag{3}$$

for the region $(W - 1) > 0.01$ and the coefficients are given in Table I. We also give the maximum variation between the curves and the results predicted from eq 3. It is clear that the use of eq 3 is a less accurate means of estimating τ_R than the curves. However, the fact that $b \cong 1$ (except for the anomalous curve F, which is presumably affected by overlap) is consistent with our interpretation of $(\Delta_i - \Delta_r^i)$ as a lifetime broadening. The curves in Figure 2 which differ only in the model of rotational diffusion are nearly identical for the region $(W - 1) > 0.03$. The more linear behavior for the free diffusion model probably reflects the fact that the picture of lifetime broadening by jumps out of the cone is a more accurate description when the root mean square jump angle $|\langle\epsilon^2\rangle_{av}|^{1/2}$ is greater than Ω the cone angle. The Brownian motion model yields curves, which, at longer τ_R's approach the rigid limit $(W - 1)$ values more rapidly than τ_R^{-1}, implying a breakdown of the simple picture.

An experimental check of this proposed method and its limitations is planned.

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(6) In fact the naive analogy to the problem of chemical exchange between two lines (*cf.* P. W. Anderson, *J. Phys. Soc. Jap.*, **9**, 316 (1954); H. M. McConnell, *J. Chem. Phys.*, **28**, 430 (1958) is a useful one. Thus, in this well-known case, in the slow-exchange limit, one has that the *A*th line with exchange-limited lifetime τ_A is broadened by τ_A^{-1}. Furthermore, the shift of the line, in a two line case, simply depends on the dimensionless product τs where $2s$ is the separation between the peaks, and $\tau^{-1} \equiv \tau_A^{-1} + \tau_B^{-1}$. In fact, when $\tau_A = \tau_B$, one has the simple result that for small τ^{-1}, the separation of the lines is decreased to $2s(1 - 2(\tau s)^{-2})^{1/2} \simeq 2s[1 - (\tau s)^{-2}]$. The result of ref 2a that $S = S(\tau_R A_z)$ (*i.e.*, it scales as the product $\tau_R A_z$) is seen to be closely related to this well-known result.

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(11) One has for random rotational reorientation that the mean time τ required to reorient by an angle Ω is given by $\tau^{-1} = 6R/\Omega^2 = [B_2\tau_R\Omega^2]^{-1}$ (*cf.* ref 7 and references contained therein). But this expression does not appear to be completely applicable to the results obtained except that $\tau^{-1} \propto \tau_R^{-1}$.

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