Dynamics of macromolecule spin-labelled side-chain groups by electron paramagnetic resonance spectra simulation

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A new approach to simulating and deciphering experimental EPR spectra of spin-labelled macromolecules is herein described. This approach is based on the fact that every EPR spectrum corresponds to the existing stationary stochastic process of angular reorientations of the aminoxyl† relative to the macromolecule to which the label is bound. Slow isotropic motion of the carrier may be described by Brownian diffusion, while the nature of the fast stochastic process of the spin label is unknown and the only way to describe it is by substituting and comparing it with well defined model processes. The general applicability of this approach is exemplified by EPR spectra simulations for various types of spin-labelled macromolecules in solutions or in bulk.

Introduction

Different physico-chemical methods have been applied to the fundamental problem of how to understand the structure of biopolymers and the mechanisms of their interactions. To understand the mechanism by which various proteins as well as nucleic acids function, one needs to know both the spatial structure of the macromolecules and their dynamic properties. The logical way to study biopolymers can be described as follows: space structure ⟶ dynamics ⟶ function. The dynamics of large biomolecules may be studied on three levels: large scale, medium scale and small scale. Large-scale dynamics is classified as the motion of sub-units of the molecule or motion of domains within a sub-unit. Medium-scale dynamics is classified as the motion of the side-residues within a polymer chain. Small-scale dynamics can be classified as the motion of the separate groups (*e.g.* methyl groups) within a polymer chain. Each type of dynamics is characterised by the corresponding rotational correlation time and reorientation amplitude.

The spin-label method is an indirect method of investigation. It is based on selective introduction of a special reporter group, an aminoxyl radical, into the investigated site of the macromolecule. Owing to the anisotropy of the magnetic moment of an aminoxyl, EPR spectra are exceptionally sensitive to the character of rotation as well as to the re-orientations of the aminoxyl itself at a time interval of 10^{-10} s to 10 s (when considering saturation-transfer spectroscopy).

The theory of the spin-label method is well developed. The direct EPR spectroscopy task has in practice been well completed, that is, one can easily compute any EPR spectrum, given the necessary model of the radical's motion. However, a simple way of solving the inverse EPR spectroscopy task—of deciphering the experimental EPR spectra, *i.e.* a method for selecting an adequate model of the radical's motion and its magnetic parameters—has not been clearly delineated. The purpose of this article is to suggest an easy and complete method by which to decipher the EPR spectra of various spin-labelled biological and synthetic samples, where the spin label has been introduced.

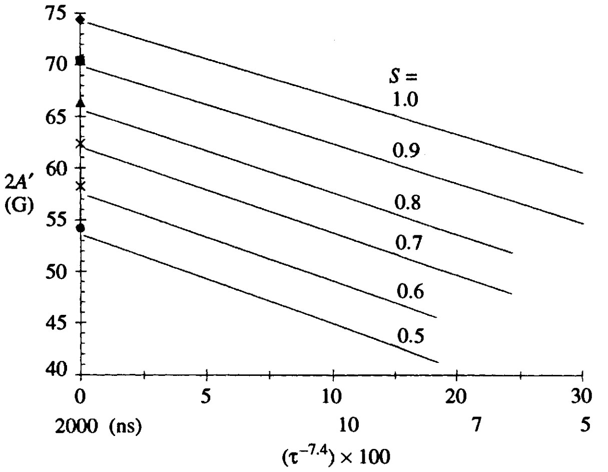

Fig. 1 Theoretical temperature–viscosity dependencies. The $2A'$-value is the separation between outer wide peaks (OWPs) in the calculated EPR spectra, S is the order parameter, and τ is the rotational correlation time. The second row of numbers under the abscissa axis corresponds to the value of τ (ns).

Results and discussion

Description of slow motion

The distance between the outer wide peaks (OWPs) in the immobilised EPR spectrum of a spin-labelled macromolecule in solution, $2A'$, differs from the maximum value $2A_{ZZ}$ obtained from a powder spectrum. Both motions of the label—slow isotropic motion together with the carrier and fast anisotropic motion relative to the latter—additionally shift the OWPs of the spectrum towards its centre. The final OWP shift can be represented by eqn. (1).[1,2] The Δ^S value, the OWP shift

$$\Delta = 2A_Z - 2A' = (2A_Z - 2\bar{A}) + (2\bar{A} - 2A') = \Delta^S + \Delta^\tau \quad (1)$$

contribution from fast reorientations of the label, will be discussed below. The addition of Δ^S and Δ^τ is possible only when the rotational correlation time of the carrier $\tau > 5$ ns, and the correlation time of the label $\tau < 0.1$ ns, and this is the situation usually achieved at ambient temperature when the viscosity of the solvent does not exceed 8 cP. The value of $2\bar{A}$ which is added and subtracted in eqn. (1) is the separation between OWPs in immobilised EPR spectra at $\tau_{\text{carrier}} = \infty$. The way[2] to obtain Δ^τ, the contribution of the slow isotopic motion of the carrier to the common shift of OWPs, Δ, will be shown below.

A set of EPR spectra has been computed with varying values of order parameter S using the correlation times τ in the range 5–2000 ns. Then OWP distances have been derived. Fig. 1 shows

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† IUPAC do not recommend the use of nitroxide to describe the $R_2N–O^\cdot$ radical. Aminoxyl should be used instead.

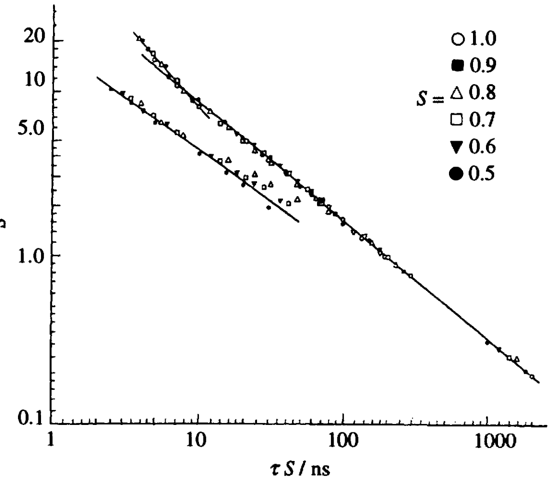

Fig. 2 Dependence of the shifts of the outer wide peaks (OWPs) (top line $\frac{\Delta^{\tau}}{S} = \frac{2\bar{A} - 2A'}{S}$) and inner wide peaks (IWPs) (bottom line $\frac{\Delta^{\tau}_{\perp}}{S} = \frac{2\bar{A}_{\perp} - 2A'_{\perp}}{S}$) on the product of the correlation time τ and the order parameter S

the dependence[1] of $2A'$ on τ and S, which in turn corresponds to the experimentally observed dependence[1] of $2A'$ on $(\eta/T)^{\frac{1}{2}}$. The same data as in Fig. 1 may be represented in Fig. 2 in a view nomogram[2] as the dependence of Δ^{τ}/S on τS, which is no longer affected by the aminoxyl type. The relation between the values τ, S and Δ^{τ} has been described before[2] and is given in eqn. (2), where the τ-value is in ns and the shift OWP Δ^{τ} is in Gauss.

$$\tau S = a(\Delta^{\tau}/S)^{b} \qquad (2)$$

The data shown in Fig. 2 may be arranged in straight lines, and one can easily derive coefficients a and b, which turned out to be $a = 197$ and $b = -1.39$ for the τ-range of 10–2000 ns and $a = 80$ and $b = -1$ for the τ-range of 5–10 ns.

Description of fast motion

Suppose that an aminoxyl label having the molecular coordinate system X,Y,Z rapidly reorients itself relative to the coordinate system X',Y',Z' fixed to the carrier or macromolecule to which the label is bound. For convenience we consider the macromolecule to be fixed relative to the laboratory coordinate system, the axis $Z_0 \| Z'$ of which is directed along magnetic field $\vec{H}$. Both $\hat{A}$ and $\hat{g}$ tensors have diagonal form in the molecular axes X,Y,Z as given by eqn. (3).

$$\hat{A} = \begin{pmatrix} A_{XX} & 0 & 0 \\ 0 & A_{YY} & 0 \\ 0 & 0 & A_{ZZ} \end{pmatrix}, \hat{g} = \begin{pmatrix} g_{XX} & 0 & 0 \\ 0 & g_{YY} & 0 \\ 0 & 0 & g_{ZZ} \end{pmatrix} \qquad (3)$$

The fast stochastic process of label reorientation with the side-chain residue of the carrier leads to partial averaging of the components of the tensors within EPR time-scales. Only the diagonal components are known and may be substituted into the final formulae of the effective spin-hamiltonian, represented in scale form. Averaging is performed by the following transformation [eqn. (4)] (all the formulae below will be written only with the components of tensor $\hat{A}$ that are analogous for the $\hat{g}$ tensor and considering that the principal axis of both tensors fit):

$$\bar{A} = \overline{T^{-1}\hat{A}T} \qquad (4)$$

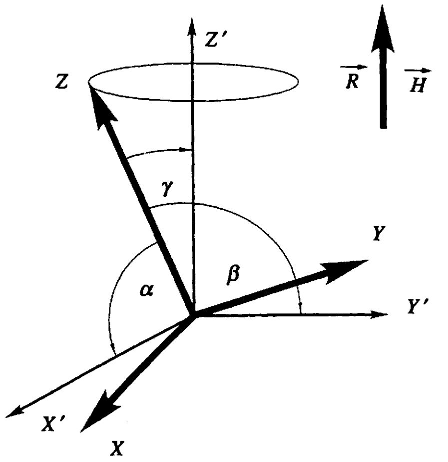

Fig. 3 Model of the rotation of the aminoxyl Z axis around the $\vec{R}$ axis, where $\alpha = \arccos \sqrt{\bar{c}^{2}_{31}}$, $\beta = \arccos \sqrt{\bar{c}^{2}_{32}}$, $\gamma = \arccos \sqrt{\bar{c}^{2}_{33}}$ (see text)

Here $\hat{A}$ is the diagonal matrix as in eqn. (3) with the trace $a_0 = (A_X + A_Y + A_Z)/3$ and T is the transformation operator from one coordinate system to another. Generally, the operator T consists of 9 directional cosines that define the X, Y, Z system position relative to that of the X', Y', Z' one, as given by eqn. (5).

$$T = \begin{pmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{pmatrix} \qquad (5)$$

The character of the stochastic process of reorientation manifests itself in the time dependence of the directional cosines. If one chooses any concrete model of fast reorientation of the aminoxyl, the averaging over time is substituted by averaging over the space angles. Many authors (for instance, Seelig[3] and Griffith[4]) discuss the simplest method of angle reorientations: rotation of the Z-axis relative to the laboratory Z'-axis with the other two axes behaving chaotically (Fig. 3). This model leads to axially symmetric tensors, which means that the c_{33} component does not depend on time. Formula (6) resulted from averaging

$$\bar{A}_{\|} = \bar{c}^{2}_{31} A_X + \bar{c}^{2}_{32} A_Y + \bar{c}^{2}_{33} A_Z \qquad (6)$$

of the parallel component of the $\bar{A}$ tensor according to eqn. (4), where the necessary condition for the direction cosines is given by eqn. (7).

$$\bar{c}^{2}_{31} + \bar{c}^{2}_{32} + \bar{c}^{2}_{33} = 1 \qquad (7)$$

The perpendicular component of the $\bar{A}$ tensor, $\bar{A}_{\perp}$, is derived under conditions that axial symmetry exists and that the trace of the tensor remains unchanged under any transformations [eqn. (8)]:

$$\bar{A}_{\perp} = \frac{3a_0 - \bar{A}_{\|}}{2} = (1 - \bar{c}^{2}_{31})A_{XX} + (1 - \bar{c}^{2}_{32})A_{YY} + (1 - \bar{c}^{2}_{33})A_{ZZ} \qquad (8)$$

It is convenient to redefine eqns. (6) and (8) using order parameters [see eqn. (9)] that comply with eqn. (10).

$$S_X = \frac{3\bar{c}^{2}_{31} - 1}{2}, S_Y = \frac{3\bar{c}^{2}_{32} - 1}{2}, S_Z = \frac{3\bar{c}^{2}_{33} - 1}{2} \qquad (9)$$

$$S_X + S_Y + S_Z = 0 \qquad (10)$$

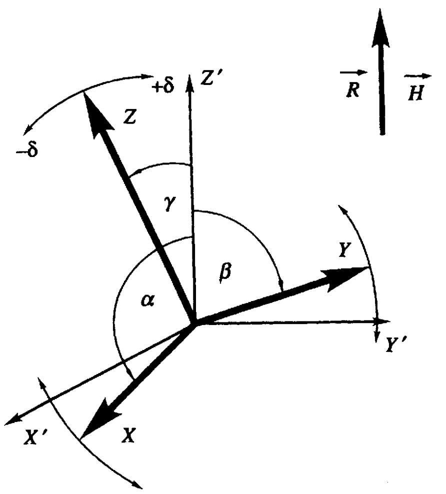

Fig. 4 Model of fast oscillations of aminoxyl around the $\vec{R}$ axis. The three angles α, β, γ (or S^R and κ, see text) define the position of the aminoxyl relative to the applied magnetic field. The arrows at the end of the XYZ coordinates show the aminoxyl oscillations from −δ to +δ.

The parallel and perpendicular components of $\overline{A}$ may now be expressed as eqns (6′) and (8′)

$$\overline{A}_{\parallel} = a_0 + \tfrac{2}{3}(S_X A_{XX} + S_Y A_{YY} + S_Z A_{ZZ}) = a_0 + \tfrac{2}{3}\overline{\Delta A} \quad (6')$$

$$\overline{A}_{\perp} = a_0 - \tfrac{1}{3}(S_X A_{XX} + S_Y A_{YY} + S_Z Y_{ZZ}) = a_0 - \tfrac{1}{3}\overline{\Delta A} \quad (8')$$

where: $\overline{\Delta A} = \Delta A S_Z - \delta A(1 - S_Z)\,\kappa$

and $\Delta A = A_{ZZ} - \dfrac{A_{XX} + A_{YY}}{2}$, $\delta A = \dfrac{A_{XX} - A_{YY}}{2}$.

If we introduce the auxiliary parameter κ, then the order parameters S_X and S_Y, as dependent on S_Z and κ, can be derived from eqn. (11), where $-1 < \kappa < +1$.

$$\begin{aligned} S_X &= \tfrac{1}{2}[-S_Z - (1 - S_Z)\kappa] \\ S_Y &= \tfrac{1}{2}[-S_Z + (1 - S_Z)\kappa] \end{aligned} \quad (11)$$

The paired parameters S and κ define a single position of the rotational axis as well as the oxcillational axis of aminoxyl in space.

According to this approach[1] the experimentally obtainable order parameter S is given by

$$S = \frac{\overline{A}_{\parallel} - \overline{A}_{\perp}}{A_Z - A_{\perp}} = \frac{\overline{A}_{\parallel} - a_o}{A_Z - a_0} \quad (12)$$

where $2\overline{A}_{\parallel} = 2\overline{A}$ and $2\overline{A}_{\perp}$ are values extrapolated from temperature–viscosity dependence (see Fig. 1) and $A_{\perp} = (A_{XX} + A_{YY})/2$ as the most probable value of S_Z, that is $S_Z = S$. All values of $\overline{A}_{\parallel}, a_o, A_Z$ are derived from the experiment as the distance between the wide (or narrow) extrema in the EPR spectra. The model of averaging described above will be called *the axial model* and includes all variants described by Griffith.[4]

One can, however, consider a more general and more complex model of X,Y,Z system oscillations with an oscillational angle α, wherein the oscillation occurs relative to the space axis $\vec{R}$ (Fig. 4). Hereafter this model will be referred to as *the oscillation model.*

In this case the operator T is expressed by matrix eqn. (13), where δ is the value of turn angle of system X, Y, Z around

$$T = \cos\delta\begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} + (1 - \cos\delta)\begin{pmatrix} c_1c_1 & c_1c_2 & c_1c_3 \\ c_2c_1 & c_2c_2 & c_2c_3 \\ c_3c_1 & c_3c_2 & c_3c_3 \end{pmatrix} + \sin\delta\begin{pmatrix} 0 & -c_3 & c_2 \\ c_3 & 0 & -c_1 \\ -c_2 & c_1 & 0 \end{pmatrix} \quad (13)$$

the $\vec{R}$ axis, with $c_1 = \cos\alpha$, $c_2 = \cos\beta$, $c_3 = \cos\gamma$ the directional cosines of the $\vec{R}$ axis, and with the corresponding order parameters given by eqn. (14).

$$S_X^R = \frac{3\,c_1^2 - 1}{2},\; S_Y^R = \frac{3\,c_2^2 - 1}{2},\; S_Z^R = \frac{3\,c_3^2 - 1}{2} \quad (14)$$

The operator defined in eqn. (13) is a sum of three matrices: a unity matrix, a symmetrical matrix and an anti-symmetrical matrix. Owing to the fact that the matrix (5) is orthogonal, the inverse matrix T^{-1} is equal to the transpose matrix $\tilde{T}$. If the former represents the sum of three matrices, then the latter is a sum of the transpose matrices. The direction cosines c_{ik} of the matrix (5) are linked to the direction cosines of the rotation axis $\vec{R}$ and the angle δ in the dependencies shown in eqn. (15).

$$c_1 = \frac{c_{32} - c_{23}}{2\sin\delta},\; c_2 = \frac{c_{13} - c_{31}}{2\sin\delta},\; c_3 = \frac{c_{21} - c_{12}}{2\sin\delta},$$
$$\cos\delta = \tfrac{1}{2}(c_{11} + c_{22} + c_{33} - 1) \quad (15)$$

Transformation of eqn. (13) as in complex (4) gives the following diagonal elements [eqns. (16)–(18)] of the matrix $\vec{A}$ averaged over the angle δ when the aminoxyl oscillates from −α to α:

$$\overline{A}_{XX} = \overline{\cos^2\delta}A_{XX} + \overline{\cos\delta(1 - \cos\delta)}2c_1^2A_{XX} + \overline{(1 - \cos\delta)^2}(c_1^2A_{XX} + c_2^2A_{YY} + c_3^2A_{ZZ})c_1^2 + \overline{(1 - \cos^2\delta)}(c_2^2A_{ZZ} + c_3^2A_{YY}) \quad (16)$$

$$\overline{A}_{YY} = \overline{\cos^2\delta}A_{YY} + \overline{\cos\delta(1 - \cos\delta)}2c_2^2A_{YY} + \overline{(1 - \cos\delta)^2}(c_1^2A_{XX} + c_2^2A_{YY} + c_3^2A_{ZZ})c_2^2 + \overline{(1 - \cos^2\delta)}(c_3^2A_{XX} + c_1^2A_{ZZ}) \quad (17)$$

$$\overline{A}_{ZZ} = \cos^2\delta\,A_{ZZ} + \overline{\cos\delta(1 - \cos\delta)}2c_3^2A_{ZZ} + \overline{(1 - \cos\delta)^2}(c_1^2A_{XX} + c_2^2A_{YY} + c_3^2A_{ZZ})c_3^2 + \overline{(1 - \cos{}^2\delta)}(c_2{}^2A_{XX} + c_1^2A_{YY}) \quad (18)$$

As is apparent, integrating values $\overline{A}_{XX}$, $\overline{A}_{YY}$ and $\overline{A}_{ZZ}$ from −α to α on the δ-scale results in only the four non-zero coefficients being preserved [eqn. (19)].

$$k_1 = \overline{\cos^2\delta} = \frac{1}{2\alpha}\int_{-\alpha}^{\alpha}\cos^2\delta d\delta = \tfrac{1}{2}\left(1 + \frac{\sin 2\alpha}{2\alpha}\right),$$

$$k_2 = \overline{\cos\delta(1 - \cos\delta)} = \frac{\sin\alpha}{\alpha} - \tfrac{1}{2}\left(1 + \frac{\sin 2\alpha}{2\alpha}\right),$$

$$k_3 = \overline{(1 - \cos\delta)^2} = 1 - 2\frac{\sin\alpha}{\alpha} + \tfrac{1}{2}\left(1 + \frac{\sin 2\alpha}{2\alpha}\right),$$

$$k_4 = \overline{(1 - \cos^2\delta)} = \tfrac{1}{2}\left(1 - \frac{\sin 2\alpha}{2\alpha}\right) \quad (19)$$

Formulae (16)–(18) could be written using the order parameters (14) as eqns. (16′)–(18′) or, using two independent parameters S_Z^R and κ, as eqns. (16″)–(18″).

$$\bar{A}_{XX} = [k_1 + \tfrac{2}{3}k_2(2S_X^R + 1)]A_{XX} + \tfrac{1}{3}k_3[a_0 + \tfrac{2}{3}(A_{XX}S_X^R + A_{YY}S_Y^R + A_{ZZ}S_Z^R)](2S_X^R + 1) + \tfrac{1}{3}k_4[(2S_Y^R + 1)A_{ZZ} + (2S_Z^R + 1)A_{YY}] \quad (16')$$

$$\bar{A}_{YY} = [k_1 + \tfrac{2}{3}k_2(2S_Y^R + 1)]A_{YY} + \tfrac{1}{3}k_3[a_0 + \tfrac{2}{3}(A_{XX}S_X^R + A_{YY}S_Y^R + A_{ZZ}S_Z^R)](2S_Y^R + 1) + \tfrac{1}{3}k_4[(2S_X^R + 1)A_{ZZ} + (2S_Z^R + 1)A_{XX}] \quad (17')$$

$$\bar{A}_{ZZ} = [k_1 + \tfrac{2}{3}k_2(2S_Z^R + 1)]A_{ZZ} + \tfrac{1}{3}k_3[a_0 + \tfrac{2}{3}(A_{XX}S_X^R + A_{YY}S_Y^R + A_{ZZ}S_Z^R)](2S_Z^R + 1) + \tfrac{1}{3}k_4[(2S_Y^R + 1)A_{XX} + (2S_X^R + 1)A_{YY}] \quad (18')$$

$$\bar{A}_{XX} = k_1A_{XX} + \tfrac{2}{3}k_2(1 - S_Z^R)(1 - \kappa)A_{XX} + \tfrac{1}{3}k_3[\Delta AS_Z^R - \delta A(1 - S_Z^R)\kappa](1 - S_Z^R)(1 - \kappa) + \tfrac{1}{3}k_4[(1 - S_Z^R)(1 + \kappa)A_{ZZ} + (2S_Z^R + 1)A_{YY}] \quad (16'')$$

$$\bar{A}_{YY} = k_1A_{YY} + \tfrac{2}{3}k_2(1 - S_Z^R)(1 + \kappa)A_{YY} + \tfrac{1}{3}k_3[\Delta AS_Z^R - \delta A(1 - S_Z^R)\kappa](1 - S_Z^R)(1 + \kappa) + \tfrac{1}{3}k_4[(1 - S_Z^R)(1 - \kappa)A_{ZZ} + (2S_Z^R + 1)A_{XX}] \quad (17'')$$

$$\bar{A}_{ZZ} = k_1A_{ZZ} + \tfrac{2}{3}k_2(2S_Z^R + 1)A_{ZZ} + \tfrac{1}{3}k_3[\Delta AS_Z^R - \delta A(1 - S_Z^R)\kappa](2S_Z^R + 1) + \tfrac{1}{3}k_4[(1 - S_Z^R)(1 + \kappa)A_{XX} + (1 - S_Z^R)(1 - \kappa)A_{YY}] \quad (18'')$$

From this general model it is very easy to analyse the specific cases. For example, if one considers the oscillations of the nitroxide from −π to π, which is the full rotation of the aminoxyl relative to $\vec{R}$ axis, formulae (16″)–(18″) can be rewritten as eqn. (20), where eqn. (21) holds.

$$\bar{A}_{XX} = a_0 + \tfrac{2}{3}(S_X^RA_{XX} + S_Y^RA_{YY} + S_Z^RA_{ZZ})S_X^R = a_0 + \tfrac{2}{3}\overline{\Delta A}^RS_X^R,$$

$$\bar{A}_{YY} = a_0 + \tfrac{2}{3}(S_X^RA_{XX} + S_Y^RA_{YY} + S_Z^RA_{ZZ})S_Y^R = a_0 + \tfrac{2}{3}\overline{\Delta A}^RS_Y^R,$$

$$\bar{A}_{ZZ} = a_0 + \tfrac{2}{3}(S_X^RA_{XX} + S_Y^RA_{YY} + S_Z^RA_{ZZ})S_Z^R = a_0 + \tfrac{2}{3}\overline{\Delta A}^RS_Z^R \quad (20)$$

$$S_X^R = (3\cos^2\alpha - 1)/2,\ S_Y^R = (3\cos^2\beta - 1)/2,\ S_Z^R = (3\cos^2\gamma - 1)/2 \quad (21)$$

All magnitudes in these formulae are expressed similarly to those in eqns. (6′), (8′) and (11) with the order parameter S_Z^R introduced in place of S_Z, for example:

$$\overline{\Delta A}^R = \Delta AS_Z^R - \delta A(1 - S_Z^R)\kappa \quad (22)$$

If κ = 0 in eqns. (20) and (22), then according to eqns. (6), (8) and (9), (11) one obtains eqns. (23) and eqn. (20) transforms to eqn. (24).

$$S_X^R = S_Y^R = -\frac{S_Z^R}{2} \quad (23)$$

$$\bar{A}_{\parallel} = a_0 + \tfrac{2}{3}\Delta A(S_Z^R)^2$$
$$\bar{A}_{\parallel} = a_0 - \tfrac{1}{3}\Delta A(S_Z^R)^2 \quad (24)$$

If one considers $\tfrac{2}{3}\Delta A = A_Z - a_o$, then the parameter S can be obtained from eqns. (22), (24) as eqn. (25) [see also eqn. (12)], that is the experimentally defined order parameter S is now equal to $(S_Z^R)^2$, the square of the order parameter of the $\vec{R}$ axis. **This is a very important result!**

$$S = \frac{\bar{A}_{\parallel} - \bar{A}_{\perp}}{A_Z - A_{\perp}} = \frac{\bar{A}_{\parallel} - a_o}{A_Z - a_o} = (S_Z^R)^2 \quad (25)$$

The second particular case involves oscillations of the XYZ coordinate system relative to the principal axis which means that one of the directional cosines c_i [see eqn. (13)] is equal to 1. Then eqns. (16″)–(18″) are correspondingly transformed into equations (26)–(28). Formulae (26)–(28) comply with the formulae derived by Griffith.[4]

if $c_1 = 1$ or $S^R = -0.5$, $\kappa = -1$, into:

$$\bar{A}_{XX} = (K_1 + 2K_2 + K_3)A_{XX} = A_{XX}$$
$$\bar{\mathrm{A}}_{YY} = K_1A_{YY} + K_4A_{ZZ} = \frac{A_{YY} + A_{ZZ}}{2} = \frac{A_{YY} - A_{ZZ}}{2}\frac{\sin 2\alpha}{2\alpha}$$
$$\bar{\mathrm{A}}_{ZZ} = K_1A_{ZZ} + K_4A_{YY} = \frac{A_{ZZ} + A_{YY}}{2} + \frac{A_{ZZ} - A_{YY}}{2}\frac{\sin 2\alpha}{2\alpha} \quad (26)$$

if $c_2 = 1$, or $S^R = -0.5$, $\kappa = +1$, into:

$$\bar{\mathrm{A}}_{XX} = K_1A_{XX} + K_4A_{ZZ} = \frac{A_{XX} + A_{ZZ}}{2} + \frac{A_{XX} - A_{ZZ}}{2}\frac{\sin 2\alpha}{2\alpha}$$
$$\bar{A}_{YY} = (K_1 + 2K_2 + K_3)A_{YY} = A_{YY}$$
$$\bar{A}_{ZZ} = K_1A_{ZZ} + K_4A_{XX} = \frac{A_{ZZ} + A_{XX}}{2} + \frac{A_{ZZ} - A_{XX}}{2}\frac{\sin 2\alpha}{2\alpha} \quad (27)$$

if $c_3 = 1$ or $S^R = 1$, $\kappa = 0$, into:

$$\bar{A}_{XX} = K_1A_{XX} + K_4A_{YY} = \frac{A_{XX} + A_{YY}}{2} + \frac{A_{XX} - A_{YY}}{2}\frac{\sin 2\alpha}{2\alpha}$$
$$\bar{A}_{YY} = K_1A_{YY} + K_4A_{XX} = \frac{A_{YY} + A_{XX}}{2} + \frac{A_{YY} - A_{XX}}{2}\frac{\sin 2\alpha}{2\alpha}$$
$$\bar{A}_{ZZ} = (K_1 + 2K_2 + K_3)A_{ZZ} = A_{ZZ} \quad (28)$$

Examples of averaging of the principal values of the aminoxyl tensors are shown in Figs. 5(*a*)–(*d*). Note that the pictures are given in pairs, *i.e.* with a fixed parameter value in one and two different values in the other. One can easily perceive the influence of either parameter on the way the tensor values average.

Modes and clusters

The introduction of clusters has already been described in detail.[5] A cluster is a multitude of aminoxyls with similar dynamic properties, *i.e.* with similar dynamic re-orientation character. Each cluster has its own set of partially averaged parameters of magnetic tensors. Each cluster is characterised by a corresponding EPR spectrum. A group of different clusters with corresponding spectra forms a more complex EPR spectrum, which can describe several specific features (fixed separation between OWPs, central peak form, *etc.*) of the experimental EPR spectrum. This complex spectrum is called spectrum mode. Finally, there may be several spectrum modes that together form the resulting spectrum Sp_{res}, which fully describes the experimental spectrum with all its peculiarities.

Simulation procedure

The principal steps for performing spectra simulation according to the present approach are described below:

1. Evaluate the principal values of the *A*- and *g*-tensors for the chosen type of aminoxyl.

2. Evaluate the rotational correlation time ($\tau = 5$–2000 ns) of the slow isotropic motion of the macromolecule.

3. Evaluate order parameter *S* for the fast ($\tau < 0.1$ ns) anisotropic re-orientation of the aminoxyl relative to the carrier.

4. Select a model for *A*- and *g*-tensor averaging and the corresponding model process for label reorientation by calculating several spectra with certain *A*- and *g*-tensor parameters with different models. The most suitable model may then be chosen by using as criteria the separation between OWPs, as well as the general shape of the spectrum.

The assumption that every cluster's EPR spectrum contributes with a 'certain weight' to the final EPR spectrum is introduced into all of the next steps. This 'certain weight' is equal to the probability that the spin labels will have some particular, partially averaged parameters of the magnetic tensors and will be in a corresponding cluster with the particular model of label re-orientations.

Hence, a cluster can be written as $Sp(S_i,\kappa_j)$ for the rotational model of averaging (at a constant value of α), or as $Sp(\alpha_n)$ for oscillation model of averaging (at constant values of *S* and κ).

5. Calculate the EPR spectra set(s) for the preferred model of averaging. Each spectrum corresponds to a cluster of macromolecules with certain partially averaged *A*- and *g*-tensor values.

Correspondingly, one has to calculate either the $(i + j)$ spectra (if rotational model has been chosen) or *n* spectra (using the oscillation model).

6. Define the spectral modes Sp^l_{ax} or Sp^m_{osc}. This step is performed by calculating the Gaussian distribution of probabilities over all possible clusters in the set [eqns. (29) and (30)] where ρ_{ij} or ρ_n is the 'weight' of each cluster's EPR spectrum in the final simulated spectrum, or the probability that a spin label will be in this particular cluster with this particular model of averaging. Consequently, a mode would be written as equation (31) or (32).

$$p_{ij} \sim e^{-\left(\frac{S_i - S_0}{\sigma_S\sqrt{2}}\right)^2} \cdot e^{-\left(\frac{\kappa_j - \kappa_0}{\sigma_\kappa\sqrt{2}}\right)^2} \quad \text{for the rotational model} \quad (29)$$

$$p_n \sim e^{-\left(\frac{\alpha_n - \alpha_0}{\sigma_\alpha\sqrt{2}}\right)^2} \quad \text{for the oscillation model} \quad (30)$$

$$Sp^l_{\mathrm{ax}} = \sum_{i,j} \rho_{ij} Sp(S_i,\kappa_j) \quad (31)$$

or

$$Sp^m_{\mathrm{osc}} = \sum_{n} \rho_n Sp(\alpha_n) \quad (32)$$

7. Simulate the spectrum that best fits the experimental spectrum by assigning 'weights' w_n to calculated modes and computing their superposition, where $w_1 + w_2 + \ldots w_n = 1$.

The resulting spectrum can be written as eqn. (33) or (34) depending on the model selected for averaging tensors.

$$Sp_{\mathrm{res}} = \sum_{l} w_l Sp^l_{\mathrm{ax}} \quad (33)$$

or

$$Sp_{\mathrm{res}} = \sum_{m} w_m Sp^m_{\mathrm{osc}} \quad (34)$$

8. When needed, repeat steps 5–8 with the other model for averaging *A*- and *g*-tensors.

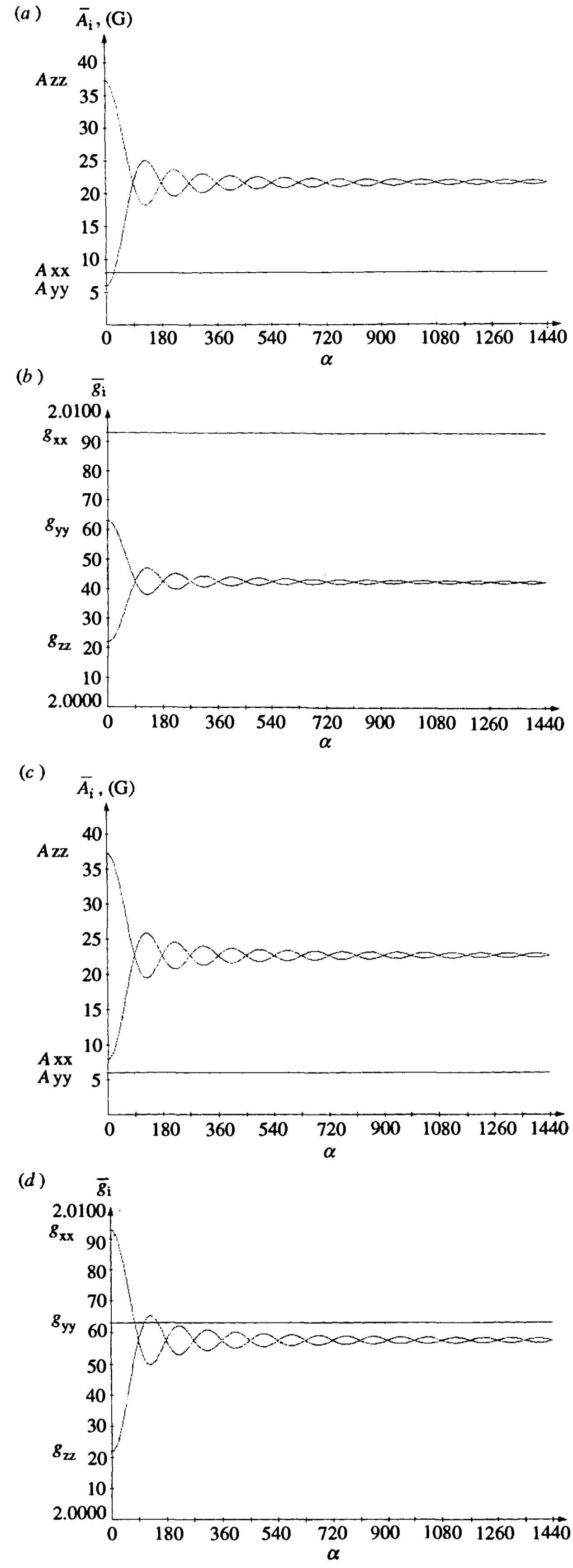

Fig. 5 Dependence of the values of the *A*- and *g*-tensor components at partial averaging on the angle of oscillation α (see text). (*a*), (*c*) Averaging *A*-tensor values; (*b*), (*d*) averaging *g*-tensor values. (*a*), (*b*) Oscillation relative to *X* axis ($S^R = -0.5$, $\kappa = -1$); (*c*), (*d*) oscillation relative to *Y* axis ($S^R = -0.5$, $\kappa = 1$).

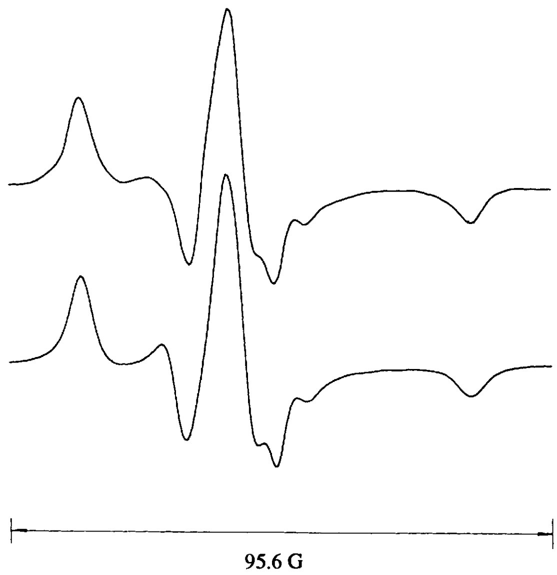

Fig. 6 Simulation of the Hg spectrum in 8% aq. ammonium sulfate. The experimental spectrum is at the top and the simulated spectrum is at the bottom. The simulated spectrum consists of one mode at $S^R = 0.57$, $\kappa = -1$ in oscillation model. Mode 1: oscillations at $\alpha_o = 56°$ with a dispersion value of $\sigma_\alpha = 7°$ and 'weight' $w_1 = 1$ (see text).

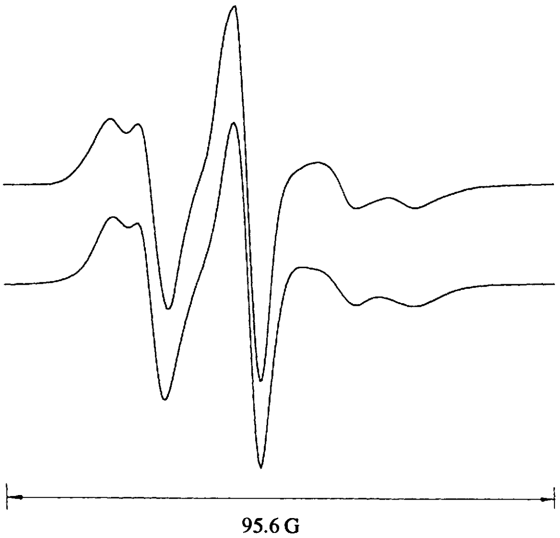

Fig. 7 Simulation of the spectrum of IgG molecule in water solution at 20 °C. The experimental spectrum is at the top and the simulated spectrum is at the bottom. The simulated spectrum consists of two modes at $S^R = 0.37$, $\kappa = -0.8$ in oscillation model. Mode 1: oscillations at $\alpha_o = 70°$ with a dispersion value of $\sigma_\alpha = 6°$ and 'weight' $w_1 = 0.87$. Mode 2: oscillations at $\alpha_o = 92°$ with a dispersion value of $\sigma_\alpha = 8°$ and weight $w_2 = 0.13$ (see text).

The final simulated EPR spectrum can then be expressed as eqn. (35) where $\mu_1 + \mu_2 = 1$.

$$Sp_{\mathrm{res}} = \mu_1\sum_l w_l Sp^l_{\mathrm{ax}} + \mu_2\sum_m w_m Sp^m_{\mathrm{osc}} \qquad (35)$$

9. Analyse the obtained data.

Examples

In order to confirm the broad possibilities of this simulation approach we applied it to three different types of molecular carriers in different media. The first sample was haemoglobin (Hb), a rigid spherical molecule. The sample was in 8% aq. ammonium sulfate at 20 °C. The label used was a piperidine-based aminoxyl with $A = (8, 6, 37.25)$ G and $g = (2.0093, 2.0061, 2.0022)$ principal tensor values with correlation time of $\tau = 300$ ns. Simulation for the Hb molecule is shown in Fig. 6. The second sample was immunoglobulin G (IgG), a flexible molecule, in water solution at 20 °C. The label used was the same as above with the same principal tensor values and a correlation time of $\tau = 26$ ns. Simulation for this molecule is shown in Fig. 7. The last sample was a dry poly(octyl isocyanate)[6] at room temperature, used to simulate the quasi-powder spectrum. The label was a pyrrolidine-based aminoxyl with tensor values $A = (7, 5, 36)$ G and $g = (2.0097, 2.0063, 2.0022)$ and correlation time of $\tau = \infty$. Simulation is displayed in Fig. 8. In all cases the simulation spectra correlated well with the experimental spectra.

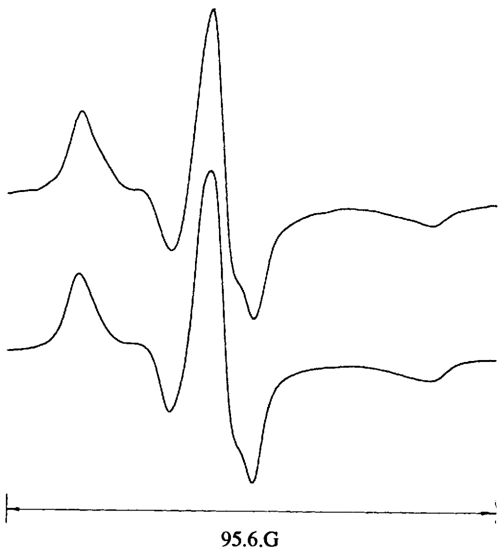

Fig. 8 Simulation of the dry poly(octyl isocyanate) spectrum. The experimental spectrum is at the top and the simulated spectrum is at the bottom. The simulated spectrum consists of three modes at $S^R = -0.1$ with κ-values for each mode of $-1, 0, +1$ corresponding to those in the oscillation model. Mode 1: oscillations at $\alpha_o = 34°$ with a dispersion value of $\sigma_\alpha = 11°$ and 'weight' $w_1 = 0.3$. Mode 2: oscillations at $\alpha_o = 32°$ with a dispersion value of $\sigma_\alpha = 14°$ and 'weight' $w_2 = 0.5$. Mode 3: oscillations at $\alpha_o = 40°$ with a dispersion value of $\sigma_\alpha = 10°$ and 'weight' $w_3 = 0.2$ (see text).

Conclusions

This present approach is applicable to all kinds of spin-labelled macromolecules and polymers. It can also be applied to spin probes[7] in macromolecules. However, its application is relatively easy and renders non-contradictory results only for immobilised spectra at ambient temperatures and small viscosities. As a rule, by the lowering temperature and raising the viscosity of the sample, it is possible to obtain a definite spectrum in immobilised form from an unknown EPR spectrum of any spin-labelled sample in triplet degenerated form at 20 °C, which can in its turn be subjected to theoretical analysis of the EPR spectra by the method proposed in this article.

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