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Continuous Diffusion Model for Concentration Dependence of Nitroxide EPR Parameters in Normal and Supercooled Water

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Abstract

Electron paramagnetic resonance (EPR) spectra of radicals in solution depend on their relative motion, which modulates the Heisenberg spin exchange and dipole-dipole interactions between them. To gain information on radical diffusion from EPR spectra demands both reliable spectral fitting for finding the concentration coefficients of EPR parameters and valid expressions between the concentration and diffusion coefficients. Here, we measured EPR spectra of the ¹⁴N- and ¹⁵N-labeled perdeuterated TEMPONE radicals in normal and supercooled water at various concentrations. By fitting the EPR spectra to the functions based on the modified Bloch equations, we obtained the concentration coefficients for the spin dephasing, coherence transfer, and hyperfine splitting parameters. Assuming the continuous diffusion model for radical motion, the diffusion coefficients of radicals were calculated from the concentration coefficients using the standard relations and the relations derived from the kinetic equations for the spin evolution of a radical pair. The latter relations give better agreement between the diffusion coefficients calculated from different concentration coefficients. The diffusion coefficients are similar for both radicals, which supports the presented method. They decrease with lowering temperature slower than is predicted by the Stokes-Einstein relation and slower than the rotational diffusion coefficients, which is similar to the diffusion of water molecules in supercooled water.

Graphical Abstract

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Supporting Information

Derivation of the EPR spectral functions of the ¹⁵N- and ¹⁴N-labeled nitroxide radicals interacting by the HSE and DD interactions, the equations for EPR parameters derived from the kinetic equation for the density matrix, and the equations for EPR parameters solved iteratively

Introduction

Electron paramagnetic resonance (EPR) spectroscopy, a sensitive technique for detecting radicals in materials, can provide information about the translational motion of radicals in a liquid solution. The relative motion of radicals modulates the Heisenberg spin exchange (HSE) and dipole-dipole (DD) interactions between them, affecting the shape of the EPR spectrum.^{1,2} The HSE interaction as a tool for finding the collision rates of radicals and their diffusion coefficients has a long history.¹ The HSE method is based on the determination of the spin exchange frequency of a radical, which is proportional to the radical concentration and the radical diffusion coefficient. This method has been applied to study the diffusion of radicals in various systems including liquids, liquid crystals, biological systems, porous hosts, etc.^{1,3–8}

Traditionally, the spin exchange frequency is obtained from the concentration-induced broadening of EPR lines, using the fact that the HSE interaction induces extra spin dephasing of the radical's magnetization, which in turn broadens the EPR lines.^{1,3–8} The line-broadening method was found to be effective at high values for the diffusion coefficient of the radical, where the contribution of HSE interactions to line broadening dominates over that of DD interactions, which is practically averaged out. As the diffusion coefficient decreases, the line broadening due to the HSE interaction decreases and the one due to the DD interaction increases, which makes the line broadening insensitive to changes in values of the diffusion coefficient. Since biologically important systems are often viscous enough to be in this regime,³ there is a need to study the concentration dependence of the other EPR parameters, as well as to separate the effects of HSE and DD interactions on measured concentration dependences. The usual way of separation in the line-broadening method relies on assumptions that the total broadening is a sum of the HSE and DD contributions that depend directly and inversely, respectively, on the diffusion coefficient whose temperature dependence follows Arrhenius behavior.^{5–8}

A more advanced approach to analyzing the experimental EPR spectra affected by the HSE interaction has been developed in a series of articles, where ref 9 is the first and ref 10 is the most recent article in the series. In the initial study,⁹ nonlinear least-squares fitting was employed for the first time to fit spin exchange broadened EPR spectra. It was shown that

the EPR line shape of a nitroxide free radical undergoing HSE in the slow exchange limit could be fit to the sum of first derivative Lorentzian absorption and dispersion lines, which is in agreement with the theoretical result of Molin et al.¹ The fitting of EPR spectra provides two additional parameters for extraction of the spin exchange frequency: (i) the ratio between the amplitudes of dispersion and absorption components, and (ii) the shifts of the resonance fields of the outer absorption lines. The amplitude ratio was found to be a linear function of spin exchange frequency,⁹ in accordance with theory.¹ The line shifts, or in other words, the absorption hyperfine spacing calculated from the line positions, was found to have one linear and one quadratic term in the spin exchange frequency.¹¹ This finding is also in agreement with theoretical considerations of the HSE-induced line shifts.¹² Additionally, a more elaborate separation method of the HSE and DD contributions was developed and applied to the stable nitroxide radical, perdeuterated 2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl (¹⁴N-pDTEMPONE) in squalane, a viscous alkane.¹³ This method uses Salikhov's theoretical analysis of the effects of HSE and DD interactions on EPR spectra,² without making any assumption on the temperature dependence of the diffusion coefficient. The separation is based on approximations applied to the sums of the spectral densities of correlation functions for the DD interaction, which define the DD contributions to the spin coherence transfer and spin dephasing.¹³ In the most recent article of the series,¹⁰ it was shown computationally that the sum of Lorentzian absorption and dispersion line shapes can be used to fit the EPR spectra of the ¹⁵N- and ¹⁴N-labeled nitroxide radicals undergoing HSE even after the individual EPR lines have coalesced and have started to narrow. Recently, Salikhov has shown theoretically that the EPR spectrum of a nitroxide at any coherence transfer rate can be expressed as a sum of Lorentzian absorption and dispersion curves.¹⁴

The above approach was also applied to study the diffusion of ¹⁴N-pDTEMPONE in the normal and supercooled states of water,¹⁵ which was the first EPR study of the translational diffusion of a nitroxide radical in supercooled water. The values of the diffusion coefficients derived from the separated HSE and DD contributions to the spin dephasing were found to be close to each other and their temperature dependences were found to follow the hydrodynamic behavior. On the other hand, the diffusion coefficient derived from the concentration dependence of hyperfine splitting was found not to follow the hydrodynamic behavior.

To further investigate this unusual behavior, we performed EPR measurements of both ¹⁴N-pDTEMPONE and pDTEMPONE labeled with ¹⁵N (¹⁵N-pDTEMPONE) in normal and supercooled water. The choice of these two radicals could be useful for testing the present approach and its further development, because the EPR spectra of these radicals sharply differ while their size and diffusion coefficient should be the same. Here, we solved the modified Bloch equations for both radicals in the presence of HSE and DD interactions. The obtained solutions were employed to fit experimental EPR spectra and extract the EPR spectral parameters for both radicals. This fitting approach, which is referred to as the original function fitting method, was compared to the fitting approach based on the sum of absorption and dispersion lines,^{9,10,14} which is referred to as the absorption-dispersion function fitting method. Using the concentration dependences of the fitted values of the EPR parameters that quantify spin dephasing, spin coherence transfer, and hyperfine splitting, we

applied linear fitting and determined the corresponding linear concentration coefficients. In the theoretical part of the study, we applied the continuous diffusion model for the relative motion of radicals. We evaluated the diffusion coefficients of radicals from the standard relations for the concentration coefficients and the relations derived by iterative solving of the kinetic equations for the spin evolution of an interacting radical pair. It was found that the latter equations predict the normal hydrodynamic behavior of the diffusion coefficients derived from the hyperfine splitting coefficients, as opposed to the standard relations. Additionally, these equations predict similar values of the diffusion coefficients calculated from all three concentration coefficients for both radicals. The temperature dependences of the calculated diffusion coefficients of the radicals were compared to the Stokes-Einstein relation and the temperature dependence of the rotational diffusion coefficient of ^{14}N -pDTEMPONE. The obtained results were compared to the temperature dependence of the rotational and translational motion of water molecules in supercooled water.

Methods

The spin probes ^{15}N -pDTEMPONE (Lot# Z447P4, 98 atom % D, 99 atom % ^{15}N) and ^{14}N -pDTEMPONE (Lot# P607P7, 99 atom % D) were purchased from CDN Isotopes and used as received. The stock solutions of 50 mM ^{15}N -pDTEMPONE and 40 mM ^{14}N -pDTEMPONE were prepared by weight in Milli-Q water. The solutions were diluted to 25 concentrations of ^{15}N -pDTEMPONE (from 2 mM to 50 mM in steps of 2 mM) and 20 concentrations of ^{14}N -pDTEMPONE (from 2 mM to 40 mM in steps of 2 mM). The samples were drawn into 5- μL capillaries (radius $\approx 150\text{ }\mu\text{m}$) and sealed at both ends by an open flame. EPR spectra were recorded with a Varian E-109 X-band spectrometer upgraded with a Bruker microwave bridge and a Bruker high-Q cavity. The sample temperature was controlled by a Bruker variable temperature unit, and it was held stable within $\pm 0.2\text{ K}$. Temperature was measured with a thermocouple using an Omega temperature indicator. The thermocouple tip was always positioned at the top of the active region of the EPR cavity to avoid reducing the cavity quality factor. All samples were measured in steps of 2 K in a temperature range from 253 to 283 K, and in steps of 4 K in a temperature range from 283 to 303 K. The EPR spectra for both probes were acquired employing a sweep time of 20 s, microwave power of 0.5 mW, time constant of 16 ms, and modulation amplitude of 0.1 G. The sweep widths of 40 and 50 G were employed for ^{15}N - and ^{14}N -pDTEMPONE, respectively.

Results

Fitting Procedure and Concentration Coefficients

The experimental EPR spectrum $S(B)$ is the first derivative of the absorption EPR signal $R(B)$ with respect to the applied magnetic field B , i.e., $S(B) = dR(B)/dB$. The modified Bloch equations for radicals with hyperfine structure in the presence of HSE can be solved in a closed mathematical form.¹ This was done for the equations of the ^{15}N - and ^{14}N -labeled nitroxide radicals interacting by both HSE and DD interactions (Appendix S1). The solution for the absorption spectra of both radicals, referred to as the original function (OF), has the form:

$$R(B)=J_0\text{Re}\left[\frac{G(B)}{1-\Lambda G(B)}\right]; G(B)=\sum_{k=1}^{2I+1}\frac{1}{z_k+\Lambda+i(B-B_0)}. \quad (1a)$$

Here, J_0 is a constant, Λ is the coherence transfer rate in magnetic-field units, B_0 is the central field position of the spectrum, and $2I+1$ is the number of hyperfine lines, where the spin of the nitrogen nucleus has values $I=1/2$ for ^{15}N and $I=1$ for ^{14}N . The parameters z_k are

$$z_1=\Gamma_1-iA/2; z_2=\Gamma_2+iA/2 \quad (1b)$$

for the ^{15}N -labeled radical, and

$$z_1=\Gamma_1-i(A+\Delta A/3); z_2=\Gamma_2+2i\Delta A/3; z_3=\Gamma_3+i(A-\Delta A/3) \quad (1c)$$

for the ^{14}N -labeled radical. In these relations, Γ_k is the spin dephasing rate or linewidth of the hyperfine line k , A is the hyperfine coupling constant, and ΔA is the relative second-order hyperfine shift.

In the OF fitting method, the first derivative of the line shape function $R(B)$ defined by eqs 1 is used to fit experimental EPR spectra. The experimental spectra were transferred to a personal computer and fitting was performed using the nonlinear regression command in the program package Mathematica. The experimental spectra for 40 mM solutions at 295 K are shown in Figure 1, together with the fitting curves and residuals. The fits are quite good, as can be seen from the residuals, and the fitted OF parameters are obtained with high precision (low standard deviation) (Table 1).

The OF fitting method is compared to the absorption-dispersion function (ADF) fitting method,^{9,10,14} which uses the fact that the absorption EPR signal (eqs 1) can be written as a sum of Lorentzian absorption and dispersion lines:¹⁴

$$R(B)=\text{Re}\left[\sum_k\frac{f_k}{z'_k+i(B-B_0)}\right]. \quad (2a)$$

Here, $f_k=f_k^R+if_k^I$ are the complex amplitudes of the hyperfine lines, whose real and imaginary parts correspond to the intensities of the absorption and dispersion components, respectively, while the parameters z'_j for the ^{15}N -labeled radical are given by:

$$z'_1 = \Gamma'_1 - iA'/2; z'_2 = \Gamma'_2 + iA'/2 \quad (2b)$$

and those for the ^{14}N -labeled radical are given by:

$$z'_1 = \Gamma'_1 - i(A' + \Delta A'/3); z'_2 = \Gamma'_2 + 2i\Delta A'/3; z'_3 = \Gamma'_3 + i(A' - \Delta A'/3). \quad (2c)$$

Here, Γ'_k , A' , and $\Delta A'$ are the linewidths, hyperfine splitting, and second-order hyperfine shift of the absorption lines.

The ADF fitting method based on eqs 2 was found to be more stable and much less time consuming than the OF method, providing the same quality of fits. However, the fitted ADF parameters are somewhat different from the corresponding OF parameters and the coherence transfer parameter Λ should be calculated from the intensities of the absorption and dispersion components (Table 1). In order to calculate the OF parameters from the fitted ADF parameters, we employ the exact relations between those parameters given in Appendix S1 (eq S1–6). The fitted OF parameters and those calculated by the exact relations exhibit a fair agreement (Table 1), which supports consistency of both fitting methods. However, the evaluation of the values and errors of the OF parameters using the exact relations was found to be somewhat inconvenient since they include equations with complex numbers. Therefore, we proposed simplified relations for the three important OF parameters: the average linewidth of hyperfine lines Γ , the coherence transfer rate Λ , and the hyperfine coupling constant A . The simplified relations are given by (see Appendix S1):

$$\Gamma = \Gamma'; \Lambda \cong \frac{A'}{2} \frac{f_2^I - f_1^I}{f_1^R + f_2^R}; A \cong \sqrt{A'^2 + 4\Lambda^2} \quad (3a)$$

for the ^{15}N -labeled radical, and by:

$$\Gamma = \Gamma'; \Lambda \cong \frac{A'}{2} \frac{f_3^I - f_1^I}{f_1^R + f_2^R + f_3^R}; A \cong \sqrt{A'^2 + 3\Lambda^2} \quad (3b)$$

for the ^{14}N -labeled radical. These relations are more practical than the exact relations, and they can be used to calculate the values and errors of the OF parameters without losing the accuracy of obtained results (Table 1).

The HSE and DD interactions between radicals affect the dependences of the EPR parameters Γ_k , Λ , and A on radical concentration.² When the radical concentration C is small enough, the parameters depend linearly on C , i.e.:

$$\Gamma_k = \Gamma_{k0} + W_{kj}C; \Lambda = \Lambda_0 + V_jC; A = A_0 - B_jC, \quad (4)$$

where W_{kj} , V_j , and B_j are the linear concentration coefficients of the k -th linewidth, the coherence transfer rate, and the hyperfine splitting, respectively. The type of radical is denoted by the index $j = 2I$, having values 1 and 2 for ^{15}N - and ^{14}N -pDTEMPONE, respectively.² The fitted OF parameters Γ_k , Λ , and A show a linear dependence on C in the measured concentration range indeed (Figure 2). Therefore, the concentration coefficients of these OF parameters can be evaluated as the slopes of the linear fits. The linear fits (Figure 2) and the corresponding concentration coefficients (Table 2) are obtained by the weighted linear regression method, where the weights are inverse squares of the standard errors from the OF fitting procedure (Table 1). We calculated the average concentration coefficients of hyperfine lines W_j , given by $W_1 = (W_{11} + W_{12})/2$ for ^{15}N -pDTEMPONE and $W_2 = (W_{12} + W_{22} + W_{32})/3$ for ^{14}N -pDTEMPONE. These average coefficients coincide with the concentration coefficients W_{Γ_j} of the average linewidths Γ (Table 2).

We also calculated the concentration coefficients of the OF parameters obtained by the exact and simplified relations from the ADF parameters (Table 2). These coefficients agree with the coefficients of the fitted OF parameters within the standard errors. Again, these results support consistency between the two fitting procedures.

By repeating the OF fitting procedure for EPR spectra and the linear regression method for the concentration dependences of the fitted parameters at each measured temperature, the concentration coefficients W_j , V_j , and B_j are obtained as a function of temperature (Figure 3). It can be seen that all three coefficients have positive values and increase with temperature in the measured temperature range. These concentration coefficients will be analyzed using the continuous diffusion model to obtain the diffusion coefficients of both radicals.

Evaluating the Effects of HSE and DD Interactions on Concentration Coefficients (Standard Relations)

In the continuous diffusion model (CDM), the relative motion of radicals A and B is characterized by the relative diffusion coefficient $D_r = D_A + D_B$, where $D_{A,B}$ are the diffusion coefficients of each single radical, respectively. The characteristic length is the distance of the closest approach of a colliding radical pair $\sigma = r_A + r_B$, where $r_{A,B}$ are the radii of the spheres representing single radicals. The characteristic time of encounter of a diffusing radical pair is given by $\tau_D = \sigma^2/D_r$, which can be understood as the total time of all reencounters of the radical pair during one encounter.¹² The HSE interaction between radicals has the form $H_{\text{HSE}} = \hbar \mathcal{J}(r) \vec{S}_A \vec{S}_B$, where the exchange integral $\mathcal{J}(r)$ is a strongly decreasing function of the relative distance r between radicals. The HSE interaction can be approximated by the exchange integral having a constant value J_0 in the narrow range of relative distances $\sigma - r - \sigma +$, where δ is a small interaction layer width. This approximation sets another characteristic time $\tau_C = \sigma^2/D_r$, which is the contact time or the total time that the radicals spend in the interaction layer during one encounter.¹² The CDM was applied for calculating the effects of DD interactions in the motional narrowing regime $\tau_D \omega_{\text{DD}} \ll 1$, where the characteristic time τ_D is much shorter than inverse of the characteristic DD frequency ω_{DD} .^{2,16–18} In this regime, the contribution of DD interaction to the EPR parameters can be written in terms of the spectral densities of the correlation

functions for the DD interaction. If, additionally, the characteristic time τ_D is much longer than the inverse Zeeman frequencies of the radicals ($\tau_D \omega_{A,B} \gg 1$), the contribution of DD interaction is only via the correlation functions of its secular part

$H_{DD}^{(0)} = \hbar \omega_{DD} (\sigma/r)^3 Y_2^0(\Omega) (S_A^+ S_B^- + S_A^- S_B^+ - 4 S_A^z S_B^z)$. Here, Ω is the orientation of relative position vector $\vec{r}$ with respect to the applied magnetic field and $\omega_{DD} = \sqrt{\pi/5} (\hbar \gamma_e^2 \mu_0) / (4\pi \sigma^3)$ is the characteristic DD frequency. Applying the above approximations to the solution of A and B radicals with respective concentrations C_A and C_B , the spin dephasing rate γ_A , coherence transfer rate λ_A , and frequency shift ω_A of A radicals due to the HSE and DD interactions with B radicals were found to be:^{2,15–18}

$$\begin{aligned} \gamma_A &= k_D C_B \text{Rep} + (C_B \kappa_{DD}^2 / k_D) \text{Re} j_\gamma \\ \lambda_A &= k_D C_A \text{Rep} + (C_A \kappa_{DD}^2 / k_D) \text{Re} j_\lambda \\ \Delta \omega_A &= k_D C_B \text{Imp} + (C_B \kappa_{DD}^2 / k_D) \text{Im} j_\gamma \end{aligned} \quad (5)$$

Here, the first and second terms are the contributions from HSE and DD interactions, respectively, $k_D = 4\pi\sigma D_r$ is the rate constant of diffusion encounters, and $\kappa_{DD} = 2\sqrt{\pi}\sigma^3 \omega_{DD}$ is the rate constant of DD interactions. The parameters p and $j_{\gamma,\lambda}$ depending on the Zeeman frequency difference $\delta = \omega_A - \omega_B$ satisfy $p(-\delta) = p^*(\delta)$ and $j_{\gamma,\lambda}(-\delta) = j_{\gamma,\lambda}^*(\delta)$. In the ¹⁵N-pDTEMPONE solution, the A and B radicals correspond to any of the two subensembles with possible frequency differences $\delta = 0, \pm a$ and concentrations $C_A = C_B = C/2$ (see eq S1-1). Using eq 5 for this solution, the concentration coefficients in G/mM units are found to be:

$$\begin{aligned} W_1 &= \frac{N_A k_D}{2\gamma_e} \text{Re} [p(a)] + \frac{N_A \kappa_{DD}^2}{2\gamma_e k_D} \text{Re} [j_\gamma(0) + j_\lambda(0) + j_\gamma(a)] \\ V_1 &= \frac{N_A k_D}{2\gamma_e} \text{Re} [p(a)] - \frac{N_A \kappa_{DD}^2}{2\gamma_e k_D} \text{Re} [j_\lambda(a)] \\ B_1 &= -\frac{N_A k_D}{\gamma_e} \text{Im} [p(a)] - \frac{N_A \kappa_{DD}^2}{\gamma_e k_D} \text{Im} [j_\gamma(a)] \end{aligned} \quad (6)$$

where N_A is the Avogadro constant and $\gamma_e = g\mu_B/\hbar$ is the electron gyromagnetic ratio (g is the radical g-factor and μ_B is the Bohr magneton). The A and B radicals in the ¹⁴N-pDTEMPONE solution correspond to any of the three subensembles with possible frequency differences $\delta = 0, \pm a, \pm 2a$ and concentrations $C_A = C_B = C/3$ (see eq S1-2). The concentration coefficients for this solution are:

$$\begin{aligned} W_2 &= \frac{N_A k_D}{3\gamma_e} \text{Re} \left[\frac{4p(a) + 2p(2a)}{3} \right] + \frac{N_A \kappa_{DD}^2}{3\gamma_e k_D} \text{Re} \left[j_\gamma(0) + j_\lambda(0) + \frac{4j_\gamma(a) + 2j_\gamma(2a)}{3} \right] \\ V_2 &= \frac{N_A k_D}{3\gamma_e} \text{Re} \left[\frac{2p(a) + p(2a)}{3} \right] - \frac{N_A \kappa_{DD}^2}{3\gamma_e k_D} \text{Re} \left[\frac{2j_\gamma(a) + j_\gamma(2a)}{3} \right] \\ B_2 &= -\frac{N_A k_D}{3\gamma_e} \text{Im} [p(a) + p(2a)] - \frac{N_A \kappa_{DD}^2}{3\gamma_e k_D} \text{Im} [j_\gamma(a) + j_\gamma(2a)] \end{aligned} \quad (7)$$

where V_2 is the coherence-transfer coefficient averaged over all pairs of the subensembles.

In the standard treatment of HSE effects, a strong exchange ($J_0 \tau_C \gg 1$), a narrow interaction layer ($\tau_C \ll \tau_D$), and a small frequency difference ($|\delta| \tau_D \ll 1$) are assumed, which gives $\text{Re } p \approx 1/2$ and $\text{Im } p \approx -(\text{sgn } \delta/4) \sqrt{|\delta| \tau_D/2}$.¹² The DD parameters are related to the spectral densities of correlation functions as $j_\gamma = 4j(0) + j(\delta)$ and $j_\lambda = 2j(0) + 2j(\delta)$, where $j(\omega) = \int_0^\infty du J_{3/2}^2(u)/(u^3 - iu\omega\tau_D)$ is the spectral density of DD interaction in the CDM.^{2,16} In the standard treatment, a small frequency difference ($|\delta| \tau_D \ll 1$) is neglected in the spectral density, which gives $j(\delta) \approx j(0) = 2/15$. Assuming that the radical solutions in our case satisfy all above conditions, the concentration coefficients (6–7) take the form:

$$\begin{aligned} W_1 &= \frac{N_A k_D}{4\gamma_e} + \frac{14N_A \kappa_{DD}^2}{15\gamma_e k_D}; V_1 = \frac{N_A k_D}{4\gamma_e} - \frac{4N_A \kappa_{DD}^2}{15\gamma_e k_D}; B_1 = \frac{N_A k_D}{4\gamma_e} + \sqrt{\frac{a \tau_D}{2}} \\ W_2 &= \frac{N_A k_D}{3\gamma_e} + \frac{38N_A \kappa_{DD}^2}{45\gamma_e k_D}; V_2 = \frac{N_A k_D}{6\gamma_e} - \frac{8N_A \kappa_{DD}^2}{45\gamma_e k_D}; B_2 = \frac{N_A k_D}{3\gamma_e} \frac{(1+\sqrt{2})}{4} \sqrt{\frac{a \tau_D}{2}}. \end{aligned} \quad (8)$$

Equations 8 are referred to as the standard relations for the concentration coefficients due to the HSE and DD interactions. We calculated the characteristic time of the encounter of a diffusing radical pair τ_D from all concentration coefficients in eqs 8. The averaged experimental values $A=22.46$ G for ^{15}N -pDTEMPONE and $A=16.04$ G for ^{14}N -pDTEMPONE were used in the calculation of the hyperfine coupling constant $a = \gamma_e A$. Since the characteristic time is related to the rate constant k_D as $\tau_D = 4\pi\sigma^3/k_D$, the only parameter that should be set in the calculation is the closest distance σ . For this parameter, we used the value $\sigma = 2r_{\text{vdW}}$, where r_{vdW} is the van der Waals radius of pDTEMPONE having the value of $3.5 \text{ \AA}$.¹⁹ We get similar values of τ_D calculated from W_j and V_j for both coefficients and both radicals, which is an expected result (Figure 4). Also, another expected result is that the values of τ_D calculated from W_j and V_j decrease with increasing temperature (Figure 4), because τ_D is inversely proportional to the relative diffusion coefficient D_r , which should increase with temperature. However, the values of τ_D obtained from the hyperfine splitting coefficients B_j are much higher than the values obtained from W_j and V_j (Figure 4). Since $B_j \propto \tau_D^{-1/2}$ is predicted by eqs 8, it follows that the standard relations, eqs 8, predict much higher B_j than is measured.

Standard relations imply that the HSE and DD contributions to the coefficient W_j can be separated by defining $H_j = (b_j W_j + V_j)/(1/j + b_j)$ and $D_j = W_j - H_j$ where $b_1 = 2/7$ for ^{15}N -pDTEMPONE and $b_2 = 4/19$ for ^{14}N -pDTEMPONE.²⁰ It can be easily shown that $H_j = W_j^{\text{HSE}}$ and $D_j = W_j^{\text{DD}}$ when eqs 8 hold. This separation allows us to calculate τ_D directly from the relative shift coefficient $\eta_j = B_j/H_j$ without any assumption about the value of σ . The values of τ_D were calculated from the relations $\eta_1 = \sqrt{a \tau_D/2}$ (^{15}N -pDTEMPONE) and $\eta_2 = (1+\sqrt{2}) \sqrt{a \tau_D/32}$ (^{14}N -pDTEMPONE), which follow from eqs 8. The values of τ_D calculated from η_j (Figure 4) are similar for both radicals, as expected, but they are much lower than τ_D obtained from W_j and V_j , which again indicates that the standard relations, eqs 8, predict much higher B_j than is measured. Additionally, the values of τ_D obtained from η_j do not decrease with temperature monotonically, but they show a maximum at about 273 K (Figure 4). This anomalous behavior has been detected for ^{14}N -pDTEMPONE in water,

suggesting that the total time of reencounters of a radical pair does not follow the hydrodynamic behavior.¹⁵

Evaluating the Effects of HSE and DD Interactions on Concentration Coefficients (Relations from the Kinetic Equations for the Spin Density Matrix)

In order to resolve the anomalous behavior that arises when the standard relations are used to calculate the concentration coefficients, we went beyond these relations by applying the formalism of the kinetic equations for the spin density matrix.^{1,12,21} This formalism, which was previously used to calculate the HSE effects on the EPR parameters within the CDM, is extended here by including the secular part of DD interactions between radicals.

We considered the kinetic equations for the spin density matrices of a system with A and B radicals with different Zeeman frequencies in an external magnetic field.^{1,12,21} Relative motion of radicals in the system is described by the CDM and the radicals interact by the HSE interaction and the secular part of DD interaction. We calculated the spin dephasing rate, the coherence transfer rate, and the frequency shift of A radicals produced by interactions with B radicals (see Appendix S2). We assume a strong HSE ($J_0 \tau_C \gg 1$) in a narrow HSE interaction layer ($x_C = r/\sigma \ll 1$), where the contact time is small enough to satisfy $|\delta| \tau_C \ll 1$ and $\omega_{DD} \tau_C \ll 1$. In this case, the parameters in eq 5 are determined by the following equations:

$$p = [p_1(\delta) + p_1^*(-\delta)]/2; j_\gamma = 2j_1 + j_2; j_\lambda = j_1 + 2j_2; j_{1,2} = [q_{1,2}(\delta) + q_{1,2}^*(-\delta)]/2; \\ p_1(\delta) = \frac{1}{4\pi} \int \frac{\partial T_1(x, \Omega)}{\partial x} \Big|_{x=1} d\Omega; q_{1,2}(\delta) = \frac{1}{i\beta_D} \int d\Omega Y_2^0(\Omega) \int_1^\infty \frac{T_{1,2}(x, \Omega) dx}{x} \quad (9)$$

where $x = r/\sigma$ ($1 < x < \infty$), $\beta_D = \omega_{DD} \tau_D$, and $T_{1,2}(x, \Omega)$ are the solutions of the equations:

$$\frac{1}{x} \frac{\partial^2 (xT_1)}{\partial x^2} + \frac{\nabla_\Omega^2 T_1}{x^2} = -i\beta_D Y_2^0(\Omega) \frac{2T_1 + T_2}{x^3} \\ \frac{1}{x} \frac{\partial^2 (xT_2)}{\partial x^2} + \frac{\nabla_\Omega^2 T_2}{x^2} + i\delta \tau_D T_2 = -i\beta_D Y_2^0(\Omega) \frac{T_1 + 2T_2}{x^3} \quad (10)$$

where ∇_Ω^2 is the angular part of Laplacian. The boundary conditions for $x = 1$ are $T_1 = T_2$ and $T_1/x = -T_2/x$, while those for $x \rightarrow \infty$ are $T_1 \rightarrow 1$ and $T_2 \rightarrow 0$. Solving eqs 10 in the first iteration (FI), the following relations for parameters in eq 9 are derived (see Appendix S3):

$$p = p_1(\delta) = p_1^*(-\delta) = \frac{1+y}{2+y} \\ j_1 = q_1(\delta) = q_1^*(-\delta) = \frac{15+10y+2y^2}{9(6+4y+y^2)}, \\ j_2 = q_2(\delta) = q_2^*(-\delta) = \frac{1}{6+4y+y^2} \quad (11)$$

where $y^2 = -i\delta \tau_D$. Equation 11 for the HSE parameter p is the same as the formula in ref 12 in the limits of a strong exchange ($J_0 \tau_C \gg 1$) and narrow interaction layer ($\tau_C \ll \tau_D$). When the HSE interaction is “switched off”, the boundary conditions for $x = 1$ are $T_1/x = -T_2/x$

$= 0$. In this case, the DD parameters in FI are $j_\gamma = 4j(0) + j(\delta)$ and $j_\lambda = 2j(0) + 2j(\delta)$, where $j(\delta) = (4+y)/(27 + 27y + 12y^2 + 3y^3)$ is the spectral density. This form of the spectral density of DD interaction has already been derived for the CDM with reflecting boundary at $r = \sigma$.²²

Inserting parameters from eq 11 into eqs 6 and 7, we calculated τ_D from all concentration coefficients for ^{15}N - and ^{14}N -pDTEMPONE, taking the same values of hyperfine constants a and closest distance σ as before. The results of the calculation show that all values of τ_D are now close to each other, especially at higher temperatures (Figure 5). Also, all values of τ_D decrease with increasing temperature without showing anomalous behavior. These results suggest that the previous specific behavior of τ_D obtained from B_j and η_j within the standard treatment is a result of the approximations employed in the standard relations (eqs 8).

Applying the second iteration (SI) to eqs 10, we calculated the corrections p , j_1 , and j_2 of the FI parameters, eqs 11. The obtained relations for the corrections have complicated forms written down in eqs S3–12 and S3–13 of Appendix S3. By adding these corrections to the FI parameters, eqs 11, and putting them into eqs 6 and 7, we calculated τ_D in SI from all the concentration coefficients (Figure 5). It can be seen that all results in the normal state above 273 K are little influenced by applying SI. Additionally, differences between the SI and FI values of τ_D are smallest for τ_D obtained from the coefficients V_j (Figure 5). Therefore, we propose the values of τ_D calculated in SI from the coefficients V_j as most reliable values of τ_D for further calculations.

Discussion

First, we analyze the relative shift coefficients η_j whose experimental values exhibit a broad maximum (Figure 6a). The values of τ_D that were directly computed from these coefficients using the standard relations, eqs 8, exhibit a similar broad maximum (Figure 4), which was referred to as the anomalous behavior of τ_D .¹⁵ On the other hand, the values of τ_D calculated in the FI and SI from eqs 6, 7, 9, and 10 exhibit normal behavior, i.e., they decrease with increasing temperature (Figure 5). Therefore, it is interesting to calculate the coefficients η_j using the SI relations for concentration coefficients and the values of τ_D calculated in SI from V_j . We can see that the coefficients η_j calculated in this way exhibit a broad maximum too (Figure 6a). This suggests again that the approximations applied in the standard relations, eqs 8, are too crude and produce the anomalous behavior of τ_D .

The second point we want to analyze is how a variation of the closest distance σ , as the only arbitrary parameter in the calculations, influences the results. For that reason we calculated τ_D in SI from the coefficients W_j , V_j and B_j at 303 K for various values of $\sigma/2$. The results show that the deviation $\tau_D(B_j) - \tau_D(V_j)$ depends on $\sigma/2$ much strongly than the deviation $\tau_D(W_j) - \tau_D(V_j)$ for both radicals (Figure 6b). This suggests that the former deviation can be used to judge the best value of $\sigma/2$. The values of $\tau_D(B_j) - \tau_D(V_j)$ are zero at about $\sigma/2 = 3.2$ Å for ^{15}N -pDTEMPONE and $\sigma/2 = 3.8$ Å for ^{14}N -pDTEMPONE (Figure 6b). The mean value of these two $\sigma/2$ are close to the van der Waals radius of 3.5 Å, which justifies our choice of $\sigma/2 = 3.5$ Å for both radicals.

Now, we can check the validity of the approximations applied in the derivation of eqs 9 and 10 for the parameters in eqs 5. First, we assumed that the DD interaction affects the EPR parameters only through the diffusion-induced modulation of its secular part. This assumption is valid when the parameter $\tau_D \omega_0$ is much larger than unity (ω_0 is the Zeeman frequency of radical). Using the facts that ω_0 is the X-band frequency (6×10^{10} rad/s) and that the calculated values of τ_D are between 500 and 2000 ps (Figure 5), it follows that in our case $\tau_D \omega_0$ is between 30 and 120, which validates our assumption. For the validation of the approximations made for HSE effects, we can take typical values $J_0 \approx 10^{11}$ rad/s for the HSE integral of the radicals at the contact distance and ≈ 0.5 Å for the HSE interaction layer width.¹³ It follows that the assumption of a narrow interaction layer is fulfilled in our case, because the relative width of interaction layer $x_C = l_C/\sigma$ has a value of about 0.07. This value of the relative width implies that the contact time $\tau_C = x_C \tau_D$ is between 35 and 140 ps. It then follows that $J_0 \tau_C$ has the values between 3.5 and 14, which agree with the assumed strong exchange limit ($J_0 \tau_C \gg 1$). Using the calculated values for the frequency difference $|\delta| \approx 4 \times 10^8$ rad/s and the DD frequency $\omega_{DD} \approx 8 \times 10^8$ rad/s, we get the values of $|\delta| \tau_C$ from 0.014 to 0.056 and the values of $\omega_{DD} \tau_C$ from 0.028 to 0.112. All these values imply that the contact time of radicals is small enough to satisfy $|\delta| \tau_C \ll 1$ and $\omega_{DD} \tau_C \ll 1$.

Finally, we calculated the translational diffusion coefficient $D_T = D_r/2$ for both radicals using the relation $D_r = \sigma^2/\tau_D$ and the values of τ_D calculated in SI from the coefficients V_j (Figure 5). The obtained results exhibit a good agreement between the values of diffusion coefficients for the two radicals with a relative deviation within 10% (Figure 7a). This result supports our method and shows a benefit of measuring all three concentration coefficients W_j , V_j , and B_j instead of only W_j , as in the line-broadening method.^{1,3–8} Temperature dependence of the translational diffusion coefficients for both radicals can be fitted to the Arrhenius law (Figure 7b) giving the activation energy values 18.43 ± 0.04 and 18.6 ± 0.3 kJ/mol for ^{15}N -pDTEMPONE and ^{14}N -pDTEMPONE, respectively.

Usually, the temperature dependence of the translational diffusion coefficient of a molecule in the normal state of liquid follows the Stokes-Einstein (SE) relation $D_T \propto T/\eta$, where η is the viscosity of liquid. The analogous relation for the rotational diffusion coefficient D_R is the Stokes-Einstein-Debye (SED) relation $D_R \propto T/\eta$. It was found that the temperature dependence of the viscosity of water in the normal and supercooled states can be well reproduced by a single power law.²³ Using this power law dependence of η , we can test the SE relation for D_T of ^{15}N - and ^{14}N -pDTEMPONE. Deviations from the SE relation can be compared to the deviations from SED relation of the measured $D_R = 1/(6 \tau_R)$ for ^{14}N -pDTEMPONE, where τ_R is the rotational correlation time.¹⁹ To do this, we define the SE ratio $R_{SE} = D_T \eta/T$ and the SED ratio $R_{SED} = D_R \eta/T$, which should be constant when the SE and SED relations are valid.²⁴ We calculated the normalized SE ratio $R_{SE}/R_{SE}(303\text{K})$ for ^{15}N - and ^{14}N -pDTEMPONE and the normalized SED ratio $R_{SED}/R_{SED}(303\text{K})$ for ^{14}N -pDTEMPONE (Figure 8a). The SE ratios increase with decreasing temperature in the supercooled state, while the SED ratio exhibits a weaker temperature dependence and a slight maximum at about 273 K.

We also calculated the normalized SE and SED ratios for D_T and D_R of water molecules, which were measured by NMR in the normal and supercooled water.^{25,26} As can be seen,

the SE ratio for water molecules increases with decreasing temperature much stronger than SED ratio (Figure 8a). It follows that both the pDTEMPONE radical and water molecule show a stronger violation of the SE relation than the SED relation in the supercooled state. This can be further illustrated by calculating the ratio D_T/D_R for ^{14}N -pDTEMPONE and water molecules.²⁶ The calculated ratios in both cases increase with lowering the temperature (Figure 8b), indicating decoupling of the translational and rotational motions in the supercooled state.^{24,26}

Conclusions

In conclusion, we measured EPR spectra for various concentrations of the ^{14}N -pDTEMPONE and ^{15}N -pDTEMPONE radicals in the normal and supercooled states of water. The EPR parameters of both radicals were calculated by fitting the spectra to the original spectral shape functions derived from the modified Bloch equations. We determined the linear concentration coefficients of the EPR parameters describing the spin dephasing, spin coherence transfer and hyperfine splitting from their concentrations dependences. To evaluate the diffusion coefficients of radicals from the concentration coefficients, we assumed that the radicals interact by the HSE and DD interactions and move according to the CDM. Taking for the closest distance between radicals, a value that corresponds to two van der Waals radii of the radical, we applied the standard relations for the concentration coefficients and the relations derived by iterative solving of the kinetic equations for the spin evolution of a radical pair. The latter equations were found to reproduce the normal hydrodynamic behavior of diffusion coefficients derived from the hyperfine splitting coefficients, as opposed to the standard relations. Additionally, these equations predict similar values of the diffusion coefficients calculated from all three concentration coefficients for both radicals. The temperature dependences of the calculated diffusion coefficients of radicals were compared to the Stokes-Einstein relation and the temperature dependence of the rotational diffusion coefficient of ^{14}N -pDTEMPONE. Upon lowering the temperature into the supercooled state, the calculated diffusion coefficients decrease slower than it is predicted by the Stokes-Einstein relation and slower than the rotational diffusion coefficient. Similar effects were detected in NMR studies of the rotational and translational motion of water molecules in supercooled water.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Experimental EPR spectra (black lines), OF fitting curves (red lines), and residuals (green lines) of 40 mM aqueous solutions of (a) ^{15}N - and (b) ^{14}N -pDTEMPONE at 295 K.

Figure 2.

Concentration dependences of the spin dephasing rates Γ_i , the coherence transfer rate Λ , and the nitrogen hyperfine splitting A for (a) ^{15}N - and (b) ^{14}N -pDTEMPONE in water at 295 K. Errors of the EPR parameters are smaller than symbols. Lines denote linear fits of the concentration dependences.

Figure 3. Temperature dependences of the concentration coefficients W_j , V_j and B_j for (a) ^{15}N - and (b) ^{14}N -pDTEMPONE in water. Errors of the coefficients are smaller than symbols.

Figure 4.

Temperature dependences of the characteristic diffusion time τ_D calculated from the standard relations (eqs 8) and the concentration coefficients W_j , V_j , B_j and η_j for (a) ^{15}N - and (b) ^{14}N -pDTEMPONE in water.

Figure 5.

Temperature dependences of the characteristic diffusion time τ_D calculated from the concentration coefficients W_j , V_j and B_j for (a) ^{15}N - and (b) ^{14}N -pDTEMPONE in water. The values of τ_D are calculated by using eqs 6, 7, 9, and the solution of eq 10 in the first (FI) and second (SI) iteration.

Figure 6.

(a) Experimental and calculated values of the relative shift coefficient η_j for ^{15}N - and ^{14}N -pDTEMPONE. (b) Deviations of $\tau_D(B_j)$ and $\tau_D(W_j)$ from $\tau_D(V_j)$ at $T=303$ K as a function of half of the closest distance $\sigma/2$ for ^{15}N - and ^{14}N -pDTEMPONE.

Figure 7. Translational diffusion coefficients of ^{15}N - and ^{14}N -pDTEMPONE in water obtained from V_j and $\sigma/2=3.5 \text{ \AA}$.

Figure 8.

(a) Normalized SE ratio $R_{SE}/R_{SE}(303\text{ K})$ and normalized SED ratio $R_{SED}/R_{SED}(303\text{ K})$ for ^{15}N -pDTEMPONE, ^{14}N -pDTEMPONE and a water molecule in water. (b) The ratio between the translational and rotational diffusion coefficients D_T/D_R for ^{14}N -pDTEMPONE and a water molecule in water.

Table 1

Fitted and Calculated Original Function (OF) Parameters and Fitted Absorption-Dispersion Function (ADF) Parameters for 40 mM Aqueous Solution of ^{15}N - and ^{14}N -pDTEMPONE at 295 K.

^{15}N OF	fitted	calculated ^a	calculated ^b	^{15}N ADF	fitted
$B_0(\text{G})$	3305.943(2)	3305.976(3)		$B_0(\text{G})$	3305.976(3)
$A(\text{G})$	21.90(2)	21.90(1)	21.90(1)	$A'(\text{G})$	21.063(6)
$\Gamma_1(\text{G})$	3.462(4)	3.458(4)		$\Gamma'_1(\text{G})$	3.458(4)
$\Gamma_2(\text{G})$	3.442(4)	3.446(4)		$\Gamma'_2(\text{G})$	3.446(4)
$\Lambda(\text{G})$	3.00(2)	3.00(2)	3.00(2)	$f_1^{\text{R}}(\text{a. u.})$	1164(3)
$J_0(\text{a.u.})$	1173(2)	1173(2)		$f_2^{\text{R}}(\text{a. u.})$	1183(3)
				$f_1^{\text{I}}(\text{a. u.})$	-356(3)
				$f_2^{\text{I}}(\text{a. u.})$	312(3)
$\Gamma(\text{G})$	3.452(3)	3.452(3)	3.452(3)	$\Gamma'(\text{G})$	3.452(3)

^{14}N OF	fitted	calculated ^a	calculated ^b	^{14}N ADF	fitted
$B_0(\text{G})$	3305.572(3)	3305.588(7)		$B_0(\text{G})$	3305.588(7)
$A(\text{G})$	15.54(1)	15.58(1)	15.58(1)	$A'(\text{G})$	15.138(9)
$A(\text{G})$	0.012(5)	0.00(2)		$A'(\text{G})$	0.00(1)
$\Gamma_1(\text{G})$	4.709(9)	4.68(1)		$\Gamma'_1(\text{G})$	4.73(1)
$\Gamma_2(\text{G})$	4.618(7)	4.59(2)		$\Gamma'_2(\text{G})$	4.50(1)
$\Gamma_3(\text{G})$	4.707(9)	4.75(1)		$\Gamma'_3(\text{G})$	4.80(1)
$\Lambda(\text{G})$	2.08(2)	2.14(2)	2.13(2)	$f_1^{\text{R}}(\text{a. u.})$	2050(10)

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¹⁴ N OF	fitted	calculated ^a	calculated ^b	¹⁴ N ADF	fitted
<i>J</i> ₀ (a.u.)	2218(8)	2209(6)		<i>f</i> ₂ ^R (a. u.)	2450(10)
				<i>f</i> ₃ ^R (a. u.)	2120(10)
				<i>f</i> ₁ ^I (a. u.)	−930(10)
				<i>f</i> ₂ ^I (a. u.)	−30(10)
				<i>f</i> ₃ ^I (a. u.)	930(10)
	4.678(5)	4.676(7)	4.676(7)	Γ' (G)	4.676(7)

^aUsing exact relations and fitted ADF parameters.

^bUsing simplified relations and fitted ADF parameters. Standard errors are given in parentheses.

Table 2

Linear Concentration Coefficients of the Fitted and Calculated Original Function Parameters for Aqueous Solutions of ^{15}N - and ^{14}N -pDTEMPONE at 295 K.

^{15}N	fitted	calculated ^a	calculated ^b
$W_{11}(\text{G/mM})$	0.0821(5)	0.0821(5)	
$W_{21}(\text{G/mM})$	0.0820(5)	0.0823(5)	
$W_1(\text{G/mM})$	0.0821(4)	0.0822(4)	
$W_{T1}(\text{G/mM})$	0.0821(5)	0.0822(5)	0.0822(5)
$V_1(\text{G/mM})$	0.0755(3)	0.0752(3)	0.0752(3)
$B_1(\text{G/mM})$	0.0150(3)	0.0151(3)	0.0151(3)

^{14}N	fitted	calculated ^a	calculated ^b
$W_{12}(\text{G/mM})$	0.1181(8)	0.1178(8)	
$W_{22}(\text{G/mM})$	0.1156(8)	0.1155(8)	
$W_{32}(\text{G/mM})$	0.1184(8)	0.1190(8)	
$W_2(\text{G/mM})$	0.1174(4)	0.1175(5)	
$W_{T2}(\text{G/mM})$	0.1174(8)	0.1174(8)	0.1174(8)
$V_2(\text{G/mM})$	0.0530(9)	0.0543(8)	0.0542(8)
$B_2(\text{G/mM})$	0.0120(2)	0.0116(2)	0.0116(2)

^aUsing exact relations and fitted ADF parameters.

^bUsing simplified relations and fitted ADF parameters. Standard errors are given in parentheses.