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Simple Test of the Effect of an Electric Field on the ^{14}N-Hyperfine Coupling Constant in Nitroxide Spin Probes

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The sensitivity of the ^{14}N-hyperfine splitting of nitroxide spin to the local electric field, $\mathbf{E}_{\mathrm{loc}}$, has often been used to probe the polarity of microenvironments; however, two uncertainties have been faced at once as follows: (i) the value of $\mathbf{E}_{\mathrm{loc}}$ in a complex microenvironment and (ii) the effect of $\mathbf{E}_{\mathrm{loc}}$ on the hyperfine splitting. In this paper, we study nitroxide free radicals that may be charged (positively or negatively) by varying the pH. The additional charge produces an additional $\mathbf{E}_{\mathrm{loc}}$, whose value we may calculate with confidence, allowing us to focus attention on the effect that $\mathbf{E}_{\mathrm{loc}}$ has on the hyperfine splitting. A simple theory is developed that provides a link between the shift in the ^{14}N-hyperfine splitting, ΔA_{N}, and the local electric field, $\mathbf{E}_{\mathrm{loc}}$. To test this approach, we have calculated the ΔA_{N} for several charged nitroxide spin probes and found good agreement (both magnitude and sign) with the measured values using $\Delta A_{\mathrm{N}} = -3.8 \times 10^{-8} E_{\mathrm{loc}}$ (ΔA_{N} in Gauss and E_{loc} in V/cm), where E_{loc} is the component of $\mathbf{E}_{\mathrm{loc}}$ directed along the N−O bond from the nitrogen to the oxygen.

I. Introduction

The optical and magnetic properties of solute molecules are strongly affected by their embedding microenvironment.[1,2] The development of a fundamental microscopic picture of the interaction of solute molecules with highly polar (dipolar, as well as charged) environments has been the focus of much research. A large number of both experimental and theoretical studies have firmly established that this interaction strongly perturbs the electronic wave function of the solute molecules, which in turn, manifests itself in highly solvatochromatic shifts in the solute absorption spectra,[3−6] as well as strongly solvent-dependent magnetic parameters, such as the isotropic nitrogen hyperfine splitting constants (A_{N}) of stable nitroxide spin probes.[7−10] It is well appreciated that a fundamental understanding at the molecular level is essential if one wants to use the measured spectroscopic parameters of a probe (solute) molecule to develop structural as well as dynamical models of the complex environments encountered, for example, in charged macromolecular or micellar fluid systems.[11] In this paper we will focus only on nitroxide spin probes and the effect of a charge-induced electric field on the ^{14}N-hyperfine splitting constant.

The literature pertaining to solute−local environment interactions is quite extensive, and here we make only a few general statements in order to inform the reader of our intent. A variety of models have been developed in order to obtain theoretically approximate wave functions of a solute perturbed by its surrounding environment, which we generically label as the *solvent*. One model is a dielectric continuum model.[12−14] In this approach the solute is represented as an electronic-state-dependent polarizable point dipole, and the solvent is described in terms of a polarizable dielectric continuum containing a spherical cavity within which the solute is located. The solute interacts with the dielectric continuum through a reaction field. The reaction field is obtained by solving either Laplace's or Poisson's equation under the appropriate boundary conditions. Another approach is to compute, by standard molecular quantum mechanical methods, the electronic structure of a *supermolecular* system consisting of the solute and solvent molecules of the first solvation shell.[15] In general, the theory of solute−solvent interactions is still one of the challenges of modern molecular quantum mechanics, and judging by the activity in this field, it is clear that much more work lies ahead.

The preponderance of the experimental and theoretical work using nitroxides has used the dielectric continuum approach, or a variation of it by adding nearby electric dipoles. In these approaches, the critical quantity is the average local electric field, $\mathbf{E}_{\mathrm{loc}}$, which is a challenging quantity to calculate.[11] Given a value of $\mathbf{E}_{\mathrm{loc}}$, workers have then been faced with the problem of calculating the effect upon the observed value of A_{N}; thus, two uncertainties have been faced at once: (1) the value of $\mathbf{E}_{\mathrm{loc}}$ and (2) the relationship of $\mathbf{E}_{\mathrm{loc}}$ with A_{N}. Our intention is to begin to disentangle these two uncertainties.

Our intention in this paper is not to generate a new theoretical model, but rather to separate the two uncertainties somewhat by applying an additional $\mathbf{E}_{\mathrm{loc}}$, whose value we may calculate to first order with confidence, thus allowing us to draw conclusions about the relationship of $\mathbf{E}_{\mathrm{loc}}$ with A_{N}. The essence of our approach is straightforward; we consider only nitroxide spin probes whose charge state (neutral or charged) can be controlled by the pH of the solution.[16−20] This charge, which is fixed in the nitroxide, gives rise to a local electric field. Classical electrostatics is used to compute the component of the electric field along the N−O bond, the region in the nitroxide where the unpaired spin density is highest and, hence, makes the largest contribution to A_{N}. We then use a simple two-level quantum mechanical model of the electronic structure of a nitroxide to provide the link between the change in the ^{14}N-hyperfine splitting constant [$\Delta A_{\mathrm{N}} = ({}^{\mathrm{charged}}A_{\mathrm{N}} - {}^{\mathrm{neutral}}A_{\mathrm{N}})$] caused by the charge fixed in the probe and $\mathbf{E}_{\mathrm{loc}}$. It should be pointed out that given the inherent uncertainties in the dielectric contiuum model, we have simplified the calculations by

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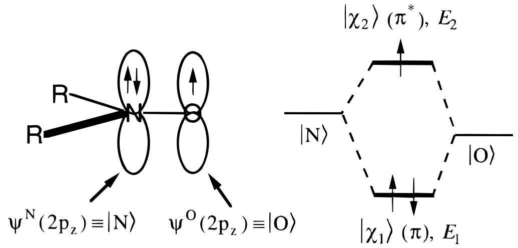


Figure 1. Simple orbital scheme in the π-plane and energy levels for a generic nitroxide spin probe.

replacing the actual charge distribution by a point charge at a point approximated from molecular models whose equilibrium geometry was determined by electronic structure calculations. We estimate the uncertainty introduced by this approximation.

This paper is organized as follows. We first give in section II an outline of the two-level model of the electronic structure of a nitroxide spin probe in order to make the connecting link between the A_N and $\mathbf{E}_{loc}$. In section III, the method of calculating $\mathbf{E}_{loc}$, within the framework of classical electrostatic theory, is described in some detail. An in-depth discussion is given in section IV on the values of the parameters used in calculating ΔA_N. We present in section V our experimental results, and finally, in section VI the calculated and experimental ΔA_N's are compared for several systems, and the merits of our approach are discussed.

II. Electronic Structure

Here we summarize the theoretical approach used to derive a simple analytical expression relating A_N to $\mathbf{E}_{loc}$; the reader interested in more details should consult the papers of Griffith *et al.*,[7] Reddoch and Konishi,[9] and Abe *et al.*[10] In addition, the reader is referred to the monograph by Mims,[21] which treats in detail the linear electric field effect on paramagnetic ions embedded in crystalline and noncrystalline hosts. For many nitroxide probes the residual spin angular momentum is highly localized in the N−O portion of the free radical. Because of this, a simple two-level quantum mechanical Hückel molecular orbital (MO) description of the electronic structure serves as a useful semiquantitative method for calculating the unpaired spin density and, thus, A_N.[22]

The one-electron Hamiltonian, which includes the perturbation due to the various internal and external fields acting on the nitroxide, is given by

$$H = H^\circ - q\mathbf{E}_{loc}\cdot\mathbf{r} \tag{1}$$

where q, the charge, is negative or positive and $\mathbf{r}$ denotes the vector from the origin to the position of the charge. The second term in eq 1 is more conveniently expressed in terms of ϕ_{loc}, the electrostatic potential produced by the embedding environment, i.e., $q\mathbf{E}_{loc}\cdot\mathbf{r} = q\phi_{loc}$. Assuming that the x-coordinate lies along the N−O bond direction, molecular orbitals with π-symmetry are constructed from linear combinations of the $\psi(2p_z)$ atomic orbital of nitrogen $|N\rangle$ and oxygen $|O\rangle$ (see Figure 1). These MOs have the form $|\chi_i\rangle = a_{i1}|N\rangle + a_{i2}|O\rangle$. Within the framework of standard Hückel theory,[23] diagonalizing the 2 × 2 matrix

$$\begin{bmatrix} \alpha_N - q\langle N|\phi_{loc}|N\rangle & k\beta \\ k\beta & \alpha_0 - q\langle O|\phi_{loc}|O\rangle \end{bmatrix} \tag{2}$$

yields the eignvectors, from which the unpaired π-electron spin density on nitrogen, $\rho_N^\pi = |a_{21}|^2$, is obtained. Here α_N and α_O are Coulomb integrals, β is the resonance integral, and k is a parameter.[23] Also, it should be noted that simplification in eq 2 is achieved because the off-diagonal matrix contributions $\langle N|\phi_{loc}|O\rangle$ are assumed small relative to the diagonal ones and were set equal to zero. We are now in a position to obtain an analytic expression for ρ_N^π as a function of $\mathbf{E}_{loc}$. First we note that diagonalization yields an expression for the coefficient a_{21}; thus

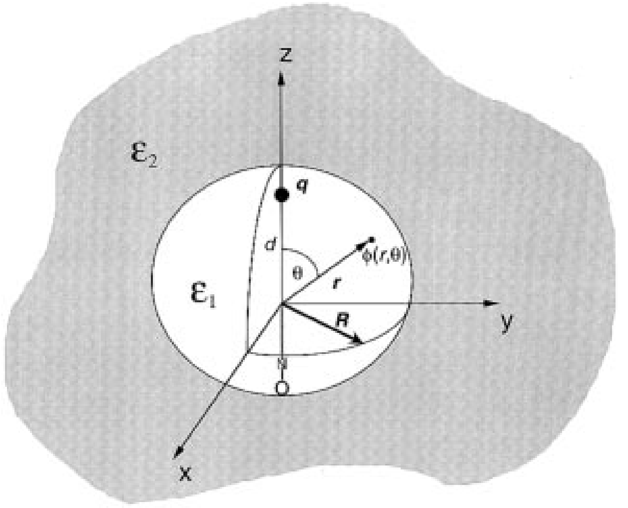


Figure 2. Coordinate system used for calculating the electrostatic potential.

$$\rho_N^\pi = \left\{1 - \left[1 + \frac{(2k\beta)^2}{(H_{NN} - H_{OO})^2}\right]^{-1/2}\right\} \tag{3}$$

where $H_{ii} = \alpha_i - q\langle i|\phi_{loc}|i\rangle$, i = N, O. Following some algebraic manipulation, we obtain

$$\rho_N^\pi \cong (1/2)[1 - (1/2)(\Delta H/k)] \tag{4}$$

where we have defined $\Delta H = (H_{NN} - H_{OO})/\beta$ and assumed that $(\Delta H)^2 << 4$. Introducing the Cohen−Hoffman[24] approximation $A_N \approx 24\rho_N^\pi + 3.6\rho_O^\pi$ and noting that $\rho_N^\pi + \rho_O^\pi = 1$, we arrive at an expression for A_N:[9]

$$A_N = 13.8 - 7.3[(\alpha_N - \alpha_O)/\beta] + 7.3q[(\phi_N - \phi_O)/\beta] \tag{5}$$

In obtaining eq 5, the heteroatom Hückel parameter k was set equal to 0.7,[23] and the $\phi_i \equiv \langle i|\phi_{loc}|i\rangle$, i = N, O, are to be interpreted as the *electrostatic potential* at nitrogen and oxygen, respectively. Further simplification can be obtained by noting that $(\alpha_N - \alpha_O)/\beta$ is a constant defined relative to the unperturbed nitroxide with a value in the approximate range −0.15 to −0.30[23] and, hence, is lumped together with 13.8 to yield ${}^{neutral}A_N$. We thus obtain the desired result:

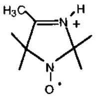
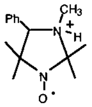
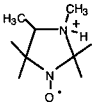
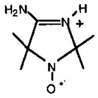
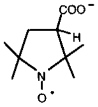
$$\Delta A_N = 7.3q[(\phi_N - \phi_O)/\beta] = -7.3qr_{N-O}E_{loc}/\beta \tag{6}$$

where r_{N-O} is the N−O bond length. In order to obtain the last term, we have used the relation $E_{loc} = -\partial\phi_{loc}/\partial r \approx -(\phi_O - \phi_N)/r_{N-O}$, where E_{loc} is the component of $\mathbf{E}_{loc}$ directed along the N−O bond from the N to the O.

III. Local Electrostatic Field Calculations

As shown above in eq 6, a value for ΔA_N can be calculated given knowledge of either $\mathbf{E}_{loc}$ or the electrostatic potentials ϕ_N and ϕ_O. Our approach here is to calculate the *electrostatic potentials* by assuming that the charge, which is localized on the spin probe, is located *at a point within the spherical cavity*. We further assume that the probe forms a homogeneous spherical cavity of static dielectric constant[7] $\epsilon_1 = 2$ embedded in a homogeneous, isotropic medium of dielectric constant $\epsilon_2 = 80$. Shown in Figure 2 is the system envisioned; R is the radius of the cavity and d locates the charge q relative to the origin.

TABLE 1: Nitroxide Probe Molecular Volume (V), Radius (R), Position Parameters (r_N, r_O, d), Angle θ, and Calculated and Observed ^{14}N-Hyperfine Shifts, ΔA_N

Probe	V/Å^3	R/Å	r_N/Å	r_O/Å	d/Å	θ/deg	ΔA_N/G*
 **RH1$^+$**	166	3.41	2.11	3.41	1.61	142	-1.02 (-0.88)†
 **RH2$^+$**	249	3.90	2.60	3.90	1.59	112	-1.27 (-1.36)†
 **RH3$^+$**	191	3.58	2.28	3.58	1.55	138	-1.06 (-1.14)†
 **RH4$^+$**	161	3.37	2.07	3.37	1.62	146	-1.01 (-0.72)†
 **CP$^-$**	175	3.47	2.17	3.47	2.92	130	+0.24 (+0.188)†

* The estimated uncertainty δ, based on a 10% variation in the parameters R, d, and θ, is $\sim\pm 0.2$ G. † Experimental $\Delta A_N/G$.

The calculation of the electrostatic potential is best treated in spherical polar coordinates, where given that the system is independent of the coordinate φ, $\phi(r,\theta)$ may be written as a linear superposition of Legendre polynomials.[25] The coefficients in the expansion are determined by the boundary conditions: (i) ϕ must be continuous across the boundary between two different media; (ii) ϕ must go to zero at infinity; and (iii) the normal component of the electric displacement must be continuous at the interface, i.e., $(\mathbf{D}_1 - \mathbf{D}_2)\cdot\mathbf{n} = 0$, where $\mathbf{n}$ is a unit vector normal to the interface.[25]

As the model is devised, $d < r_O$, where r_O is the radial distance to the oxygen atom in all cases. For four of the five nitroxides considered here, $d < r_N$ (r_N is the radial distance to the nitrogen atom), as well; however, for one, $d > r_N$. We illustrate the theory here for the case $d < r_O$ and $d < r_N$; the other case proceeds similarly. For the systems under consideration we have the following equations for the potential $\phi(r,\theta)$:

$$\phi_1(r,\theta) = \left(\frac{q}{4\pi\epsilon_0\epsilon_1}\right)\sum_{l=0}^{\infty}\left\{\left(\frac{r^l}{d^{l+1}}\right) + A_l r^l\right\}P_l(\cos\theta),\quad 0 < r < d \tag{7}$$

$$\phi_2(r,\theta) = \left(\frac{q}{4\pi\epsilon_0\epsilon_1}\right)\sum_{l=0}^{\infty}\left\{\left(\frac{d^l}{r^{l+1}}\right) + A_l r^l\right\}P_l(\cos\theta),\quad d < r < R \tag{8}$$

and

$$\phi_3(r,\theta) = \left(\frac{q}{4\pi\epsilon_0\epsilon_1}\right)\sum_{l=0}^{\infty}\left\{\left(\frac{d^l}{r^{l+1}}\right) + B_l r^{-(l+1)}\right\}P_l(\cos\theta),\quad R < r < \infty \tag{9}$$

The first term in each of the above eqs 7–9 corresponds to the potential of the source q, and the second one represents the contribution to the potential from the induced polarization. From the boundary conditions $\phi_2(R) = \phi_3(R)$ and $\epsilon_1(\partial\phi_2/\partial r)_{r=R} = \epsilon_2(\partial\phi_3/\partial r)_{r=R}$, which must hold for all values of the angle θ, we obtain

$$A_l = \left(\frac{q}{4\pi\epsilon_0\epsilon_1}\right)\left\{\frac{(\epsilon_1 - \epsilon_2)(l+1)}{[l\epsilon_1 + (l+1)\epsilon_2]}\right\}\left[\frac{d^l}{R^{2l+1}}\right] \tag{10}$$

In arriving at eq 10 we have taken advantage of the orthogonality of the functions $P_l(\cos\theta)$. Therefore, in the range of interest $d < r_\mu < R$, the potential $\phi_\mu(r_\mu,\theta)$ at either nitrogen or oxygen (μ = N, O) is given by

$$\phi_\mu(r_\mu,\theta) = \left(\frac{q}{4\pi\epsilon_0\epsilon_1}\right)\sum_{l=0}^{\infty}\left\{\left(\frac{d^l}{r_\mu^{l+1}}\right) + \left[\frac{(\epsilon_1 - \epsilon_2)(l+1)}{[l\epsilon_1 + (l+1)\epsilon_2]}\right]\left(\frac{d^l r_\mu^l}{R^{2l+1}}\right)\right\}P_l(\cos\theta) \tag{11}$$

Equation 11 is the basic expression used for calculating the electrostatic potentials $\phi_N(r,\theta)$ and $\phi_O(r,\theta)$,[11] which are used in eq 6 to calculate ΔA_N. As pointed out above, for one of the nitroxides investigated, r_N falls in the range $0 < r_N < d$. In order to calculate the electrostatic potential $\phi_N(r_N,\theta)$ for this radical, the first term in eq 11 needs to be inverted, i.e., $(d^l/r_\mu^{l+1}) \rightarrow (r_\mu^{l+1}/d^l)$. In the following section we describe the rationale used to assign values to r_N, r_O, d, R, and θ.

IV. Parameter Assignment

A. r_μ, d, R, and θ. In order to assign physically realistic values to r_μ, d, R, and θ (see Figure 2), we need to examine the molecular structure of the various nitroxide probes studied. Listed in Table 1 are the structural formulas of the five nitroxide probes (ionic form) that were considered. Before embarking

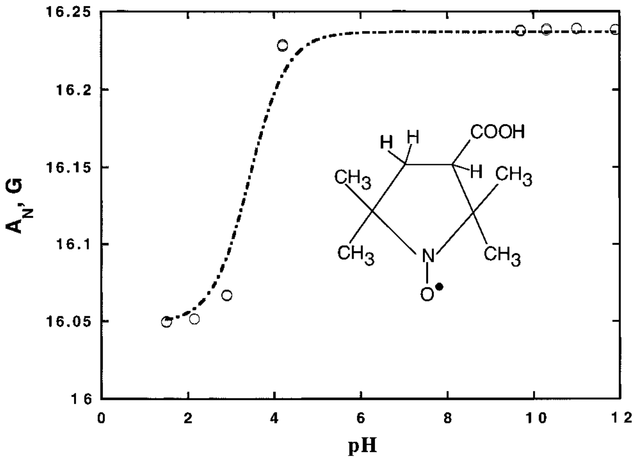


Figure 3. Plot of A_N *versus* pH for the CP radical. The open circles are the experimental data, and the broken curve is a least-squares fit to the Henderson−Hasselbalch equation.

on this discussion let us recapitulate that r_μ, d, R, and θ are referenced to a coordinate system whose origin is located at the center of a sphere having dielectric constant $\epsilon_1 = 2$. This dielectric sphere is conceptually generated by envisioning that in the fluid state the nitroxide is undergoing rapid *isotropic* rotational diffusion, thus leading to the displacement of solvent molecules in its immediate vicinity. The *radius* of the spherical volume occupied by the tumbling probe, that is, the value of R, can be approximately obtained from van der Waals *volumes* (V) of the nitroxides. The volumes were computed by using Bondi's[26] method and are listed in Table 1 along with the values of R [$=(3V/4\pi)^{1/3}$] for the five probes. Space-filled models of the probes were also generated from electronic structure calculations that utilized geometry optimization techniques. The electronic structure calculations were used for other purposes to be discussed below; the molecular volumes were just the convenient by-product of the calculations. The probe volumes obtained from electronic structure calculations were in excellent agreement with those determined by Bondi's method.

Since the oxygen of the N−O moiety is located at the interface, we assume that $r_O = R$. For r_N we have $r_N = R - r_{N-O} = R - 1.3 \times 10^{-8}$ cm, where r_{N-O} is the N−O bond length and assumed to be ~1.30 Å (average value of r_{N-O} for a variety of nitroxides[27]).

The computed electronic structure of the various spin probes was used to determine the distance parameter d and the angle θ. MOPAC (Molecular Orbital Package),[28] a system of semiemperical SCF-LCAO-MO molecular quantum mechanical programs for solving the Roothaan−Hall equations,[29] was used to compute the optimized geometry and electronic properties of the nitroxides. The effects of the highly polar aqueous environment were taken into account by using programs modified to include a conductor-like screening model (COSMO),[30] i.e., a technique that approximates the dielectric screening energy of a solvent by the method of image charges. From an analysis of the partial charge distribution in the molecule, information about the location of the excess charge was ascertained. Once this was in hand, both the angle θ and the distance d to the origin of the coordinate system located at the approximate center-of-mass (center of the sphere) of the nitroxide could be found. Tabulated in Table 1 are the parameters r_N, r_O, R, d, and θ used in eq 11 for calculating the electrostatic potentials $\phi_N(r,\theta)$ and $\phi_O(r,\theta)$.

B. The Hückel parameter $\boldsymbol{\beta}$. In order to calculate ΔA_N using eq 6, a value for β must chosen. This was accomplished by relating the optical absorption spectrum to the energy difference $\Delta E_{1,2}$ of the $\pi \rightarrow \pi^*$ transition of the nitroxide group.[31,32] From simple Hückel theory we have the relation

$$\Delta E_{1,2} = -[h^2 + 4(k')^2]^{1/2}\beta \qquad (12)$$

which is obtained by diagonalizing eq 2, with the following modifications: replace α_N by $\alpha_O + h\beta$ and $k\beta$ by $k'\beta$ and set $\phi_{loc} = 0$. Assuming $h \approx -0.5$ and $k' \approx 1$[7,23] and that the observed intense UV absorption at $\lambda_{peak} \approx 240$ nm is assigned to the $\pi \rightarrow \pi^*$ transition localized on the N−O moiety,[31,32] we obtain $\beta \approx -2.5$ eV.

An important issue here is the assignment of the observed 240 nm optical band to a $\pi \rightarrow \pi^*$ transition. In order to confirm this, we have computed the electronic absorption spectrum of the nitroxide spin probe RH1$^+$ using the semiempirical modified intermediate neglect of differential overlap (INDO) method,[28,29] including configuration interaction (CI).[29] The computed absorption spectrum reveals a strong component around ~230 nm, and a display of the wave functions active in the transition confirms the π-symmetry of the orbitals, which are localized in the N−O bond region.

V. Experimental Results and Discussion

We have taken from the literature[16,17] measurements of ΔA_N for neutral/cationic forms of nitroxide free radicals and have undertaken measurements of the anionic/neutral forms of the nitroxide 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxyl (CP). Other results are also available in the literature.[18,19] CP was purchased from Aldrich and used as received. Solutions were prepared in doubly distilled water, by weight, to a concentration of 5×10^{-5} M. The pH was varied by adding NaOH or HCl solutions and was measured with an Accumet Model 5 pH meter. The samples, not degassed, were sealed in 50 μL

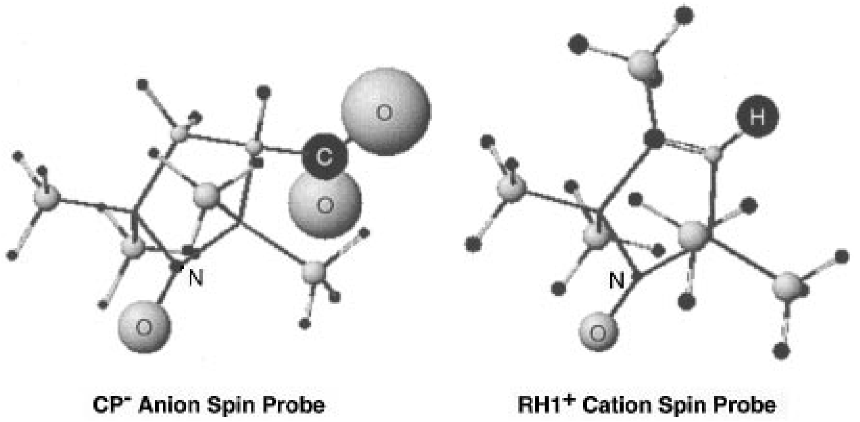


Figure 4. Calculated partial charges for the CP$^-$ radical anion and RH1$^+$ spin probes. The light spheres represent excess negative charge, whereas excess positive charge is depicted by dark spheres. See text for details.

disposable pipets. The EPR measurements were carried out at room temperature (23 ± 2 °C) using a Bruker 300 ESP E X-band spectrometer employing 100 kHz field modulation. The magnetic field sweep was calibrated with an ER 035 M NMR gaussmeter.

Three-line, narrow EPR spectra, typical of nitroxide free radicals undergoing rapid, isotropic motion,[34] were observed at all values of the pH. The value of A_N was taken to be one-half the difference in the resonance fields of the high- and low-field lines. These resonance fields were found by fitting the observed spectra to a Gaussian−Lorentzian sum function as previously described.[35] Using these fits, A_N is reproduced to better than ±0.001 G. Absolute values of A_N are estimated to be accurate to ±0.01 G.

Figure 3 shows the variation of A_N with pH. The solid line is a least-squares fit of the Henderson−Hasselbalch equation to the data assuming that the values of A_N result from an averaging of the hyperfine coupling constants in the neutral and anionic forms that is fast compared with $\gamma\Delta A_N$, where $\gamma = 1.76 \times 10^7$ s^{-1} G^{-1} is the gyromagnetic ratio of the electron. The estimated pK_a is 3.4 ± 0.2. If, in fact, the averaging is slow, the apparent pK_a may be shifted somewhat.[18] At pH 1.5, $A_N = 16.049$ G, and at pH = 11.9, $A_N = 16.237$ G, yielding $\Delta A_N = +0.188 \pm 0.002$ G. The fact that A_N is rather insensitive to H$^+$ concentration over several orders of magnitude shows that the counterion has a negligible effect upon ΔA_N. This is to be expected since the rapid tumbling of the nitroxide will effectively average the electric field produced by an ion in solution. In experiments utilizing added NaCl, we found that the change in A_N was less than 0.02 G per mole of added salt, showing that the electric field due to these ions is also effectively averaged.

VI. Theoretical Results and Discussion

An encapsulated view of the results of our methodology is presented in Table 1. It is quite clear from the data given in this table that the results are quite impressive. The experimental magnitudes and signs of ΔA_N compare well with theory. The rather subtle differences in the distances between the effective charges and the N−O bond in the various neutral/cationic radicals are reflected in the variation of ΔA_N.

In Figure 4 we show the calculated partial charges for the carboxy anion radical (CP$^-$) and RH1$^+$. The partial charges were calculated using the semiemperical SCF-LCAO-MO molecular quantum mechanical software described in section IV and represent the excess positive or negative charge relative to the neutral atom. A *dark* sphere indicates *positive* charge, whereas the *light* spheres represent *negative* charge; the radii of the sphere is proportional to the magnitude of the charge. The scale factor is the same for both displayed radicals. In principle the partial charges of the neutral radicals should also be shown for comparison; however, the point here is to provide one with a pictorial aide for visualizing where the excess charge is concentrated.

For CP$^-$ the excess negative charge builds up in the carboxyl group, as anticipated; and for RH1$^+$ the positive charge is localized on the proton attached to the nitrogen atom in position 3 (the nitrogen in the N−O bond is designated as position 1; a counterclockwise numbering scheme is used to label the other atoms). These results provide justification for using a point charge approximation for evaluation of the electrostatic potential $\phi_\mu(r_\mu,\theta)$ at either nitrogen or oxygen (μ = N, O) as described in section III.

In addition, from calculated electron density maps one observes that the carboxyl group protrudes significantly from the surface of the molecule and is located, in our simple picture, at the interface between the two dielectric regions. This means that one should expect a large value for d when compared to the other radicals such as RH1$^+$. As indicated in Table 1, we see that the CP$^-$ does indeed have the largest d.

In summary, we have examined the validity of a simple theory that provides a link between the shift in the ^{14}N-hyperfine splitting, ΔA_N, and the local electric field, $\mathbf{E}_{loc}$. We have used a simple classical electrostatic model to calculate $\mathbf{E}_{loc}$, which is a consequence of microenvironmental perturbations such as those anticipated in charged micellar systems. From the good agreement found between the calculated and measured ΔA_N for several charged nitroxides we conclude that this approach should prove useful for interpreting the electron paramagnetic spectrum of spin probes in complex charged environments.

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