Homonuclear Overhauser Enhancements as Probes of Molecular Mobility

L. G. Werbelow

Contribution from the Chemistry Department, University of British Columbia, Vancouver 8, Canada. Received January 28, 1974

Abstract: Detailed calculations of homonuclear Overhauser enhancements (NOE's) for a pair of dipolar relaxed nuclei in the motional limit of symmetric top reorientation (overall isotropic mobility, one degree of internal mobility) are presented. The useful range of motional times probed by this technique is analogous to that probed by the usage of T_1/T_2 or $T_2(\omega_o)/T_2(\omega_o')$ ratios; yet the information obtained from such investigations is distinctly unique. The results obtained differ considerably from a similar computational study for heteronuclear enhancements. It is concluded that, *in general*, a measurement of the Overhauser enhancement is insensitive to the *internal* mobility and depends solely on the overall mobility.

In recent years, the nuclear Overhauser effect (NOE) has proven to be a very powerful tool for varied chemical investigations.[1] Thus far, the primary usage has been restricted to molecular structural and identification studies and for the characterization of various relaxation pathways and related problems in nuclear magnetic relaxation.

As an indirect consequence of the recent trend toward biological nmr studies, another usage of the NOE emerges, as it provides a novel method to obtain the quantities parameterizing the dynamics of molecular processes.

Interest in this facet of the NOE was aroused by recent homonuclear Overhauser studies performed by Bothner-By and coworkers.[2] Relevant discussions of the motional usage of the NOE and experimental examples can be found in these and related papers.

Theory

Consider an ensemble of spin systems composed of two nonequivalent spin $1/2$ nuclei subjected to a large Zeeman field (the ensemble being in nonequilibrium with its surroundings). It can easily be derived from a Solomon-type treatment, or a more sophisticated density matrix treatment, that the time dependence of the deviation magnetizations of the two spins

$$I_\xi \equiv \langle I_z \rangle - \langle I_z \rangle^{\text{thermal}} \quad (1a)$$

$$S_\xi \equiv \langle S_z \rangle - \langle S_z \rangle^{\text{thermal}} \quad (1b)$$

obey the equation of motion

$$\dot{\Xi} = -\Phi\Xi \quad (2)$$

where

$$\Xi \equiv \begin{pmatrix} I_\xi \\ S_\xi \end{pmatrix}$$

and

$$\Phi \equiv \begin{pmatrix} 1/T_1^I & 1/T_1^{IS} \\ 1/T_1^{SI} & 1/T_1^S \end{pmatrix}$$

The expressions for the elements of Φ are

$$1/T_1^I \equiv {}^{1}/_{3}J_0(\omega_I - \omega_S) - J_1(\omega_I) + 2J_2(\omega_I + \omega_S) \quad (3a)$$

$$1/T_1^{IS} \equiv -{}^{1}/_{3}J_0(\omega_I - \omega_S) + 2J_2(\omega_I + \omega_S) \quad (3b)$$

(1) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect," Academic Press, New York, N. Y., 1971.
(2) P. Balaram, A. A. Bothner-By, and E. Breslow, *Biochemistry*, **12**, 4695 (1973); *J. Amer. Chem. Soc.*, **94**, 4015 (1972).

with T_1^{SI} and T_1^S obtained upon the exchange of the appropriate indices. Assuming only intramolecular dipolar interactions, the various spectral densities appearing in eq 3 are given by

$$J_k(\omega) = (6\pi/5)(\gamma_I\gamma_S\hbar/r_{IS}^3)^2 \times$$
$$\int_0^\infty \langle Y_2^k(\Omega(\tau))Y_2^{-k}(\Omega(0))\rangle \exp(i\omega\tau)d\tau \quad (4)$$

The unique solution to eq 2 is dependent upon the initial preparation of the spin system, a facet of the nmr experiment left to the discretion of the experimentalist. In this note, we wish to consider only the classic steady state nuclear Overhauser effect. Assuming spin S is saturated and the intensity of spin I is observed, the steady state boundary conditions are

$$\dot{\Xi} = 0 \quad (5a)$$

$$\Xi = \begin{pmatrix} I_\xi \\ \overline{\langle S_z \rangle^{\text{thermal}}} \end{pmatrix} \quad (5b)$$

The fractional enhancement factor ($\eta = I_\xi^{\text{steady state}}/\langle I_z \rangle^{\text{thermal}}$) of the observed signal can now be derived from the solution of eq 2 subject to the stipulated conditions of eq 5

$$\eta = (\gamma_S/\gamma_I)(J_0(\omega_I - \omega_S)/3 - 2J_2(\omega_I + \omega_S))/(-J_0(\omega_I - \omega_S)/3 + J_1(\omega_I) - 2J_2(\omega_I + \omega_S)) \quad (6)$$

Obvious from eq 6 is the fact that η is dependent upon the ratio of unlike combinations of spectral densities, a fact most promising for those seeking microscopic motional information from a macroscopic nmr observable. Further inspection shows that if the spectral density is neither black nor white (which is often the case for biologically interesting molecules at present experimental field strengths) then, indeed, the measurement of η will in principle yield a tool to quantitate molecular mobility. As might be expected, the usage of such information is in many ways analogous to the information gathered from the more familiar spectral density ratios such as T_1/T_2 ratios, $T_2(\omega_o)/T_2(\omega_o')$ ratios, or $T_1(\omega_o)/T_1(\omega_o')$ ratios.

Unfortunately, deciphering the nmr macroscopic observables into a detailed (or even meaningful) description of molecular mobility is often clouded and ambiguous and hence must be approached with care. Discussion of this problem has repeatedly appeared in
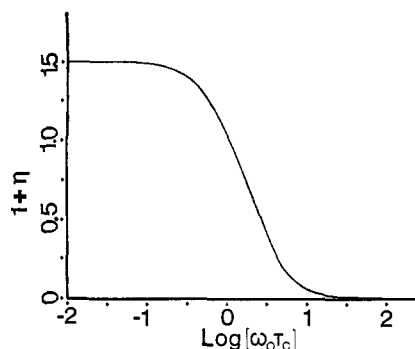
Figure 1. Plot of the normalized NOE fractional line intensity, $1 + \eta$, as a function of *isotropic* mobility of a pair of homonuclear spins relaxed by dipolar interactions. $\tau_c \equiv 1/6D$ where D, the rotational diffusion constant, is measured in units of $\omega_0/6$.

Figure 2. Contour (topographical) plots of the normalized NOE fractional line intensity, $1 + \eta$, as a function of both overall isotropic mobility and internal flexibility. τ_c is defined as in Figure 1 and $\tau_R = 1/D_{int}$ where D_{int} (in units of ω_0) quantifies the one-dimensional internal rotation. The dependence of the direction of the internuclear vector with respect to the internal rotor axis is depicted for three angles and explained in the discussion. Contours are shown for $\eta + 1 = 0.05, 0.25, 0.75, 1.25,$ and 1.45.

the literature in connection with interpretation of relaxation times and ratios of relaxation times.[3] We shall now extend these considerations to the interpretation of nuclear Overhauser enhancements.

The simplest model of molecular mobility (aside from the trivial example of isotropic reorientation for the pair of spins) is to consider two spins attached to an internal rotor, which is itself rigidly attached to an isotropically reorienting molecular framework. The internuclear vector defines an angle β with respect to the axis of internal rotation.

Using the theory of rotational Brownian motion Hubbard[4] has given explicit expressions for the one-sided Fourier transform of the autocorrelation function of the second rank spherical harmonics appearing in eq 4

$$\int_0^\infty \langle Y_2^k(\Omega(\tau)) Y_2^{-k}(\Omega(0)) \rangle \exp(i\omega\tau) d\tau =$$

$$(-1)^k/(16\pi)\{(3 \cos^2 \beta - 1)^2 F_0(D, D_{int}, \omega) +$$

$$12(\sin \beta \cos \beta)^2 F_1(D, D_{int}, \omega) +$$

$$3 \sin^4 \beta F_2(D, D_{int}, \omega)\} \quad (7)$$

where $F_n(D, D_{int}, \omega) = (6D + n^2 D_{int})/((6D + n^2 D_{int})^2 + \omega^2)$. D is the rotational *diffusion* constant characterizing the isotropic overall motion and D_{int} is the rotational *diffusion* constant characteristic of the one-dimensional internal rotation.[5] (It is a simple matter to convince oneself that eq 7 can also be interpreted as describing a pair of spins *rigidly* attached, at an angle β, to a hydrodynamic, prolate ellipsoid with the redefinitions $D \rightarrow D_\perp$ and $D_{int} \rightarrow D_\parallel - D_\perp$, where $D_\perp$ and $D_\parallel$ are the conventional parameters characteristic of symmetric top rotational diffusion.) Before presenting numerical solutions to eq 6, we first proceed to an important limiting form of this equation.

Doddrell, *et al.*,[6] have presented a similar treatment

(3) For example: L. G. Werbelow and A. G. Marshall, *J. Amer. Chem. Soc.*, **95**, 5132 (1973); A. G. Marshall, P. G. Schmidt, and B. D. Sykes, *Biochemistry*, **11**, 3875 (1972).

(4) P. S. Hubbard, *J. Chem. Phys.*, **51**, 1647 (1969).

(5) Actually the treatment presented here is much more general than implied by eq 7. In a very general calculation, using only the symmetry properties of spherical tensors under rotations, P. S. Hubbard, *Phys. Rev.*, **180**, 319 (1969), has shown that the left-hand side of eq 7 for various k are in fixed ratios and requires no *a priori* model (diffusional, etc.) for reorientation. Also, the small imaginary second-order corrective terms in eq 2 are omitted as they can be shown to have a negligible effect on these calculations for all valid values of D ($D \gg$ rigid lattice line width).

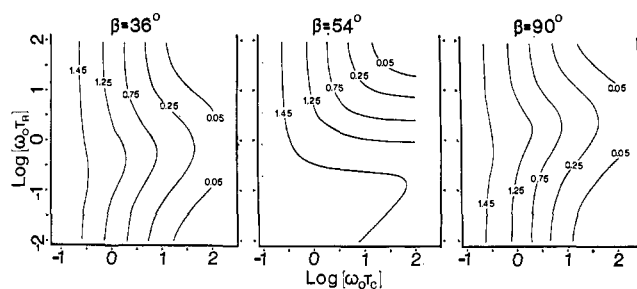(6) D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, **56**, 3683 (1973).

to the problem outlined in this section, and except for changes in formalism it is completely equivalent. However, their results were specialized to a computational *hetero*nuclear NOE study where spin S was a proton and spin I a ^{13}C nucleus. In the specialized case considered here, it is assumed that the two nuclei are of the same species ($\gamma_I = \gamma_S$) but that their resonant frequencies are separated by at least a few line widths. The validity of the approximations

$$J_0(\omega_I - \omega_S) \cong J_0(0) \quad (8a)$$

$$J_1(\omega_I) \cong J_1(\omega_S) \equiv J_1(\omega_0) \quad (8b)$$

$$J_2(\omega_I + \omega_S) \cong J_2(2\omega_0) \quad (8c)$$

suffices to computationally define the homonuclear approximation as used in this paper.

Discussion

In Figure 1, the observable normalized fractional line intensity, $1 + \eta$, is plotted for a pair of spins reorienting isotropically (*i.e.*, where the motion is completely characterized by a single diffusion constant). Throughout this paper, we shall identify $\tau_c \equiv (6D)^{-1}$. To present the results in a general fashion which is independent of a particular experimental Zeeman field, the dimensionless quantity $\omega_0\tau_c$ is chosen as the unit of mobility.

The asymptotic values in both the fast motion regime ($\omega_0\tau_c \ll 1$; $J_0(0) \cong |J_1(\omega_0)| \cong J_2(2\omega_0)$) and slow motion regime ($\omega_0\tau_c \gg 1$; $J_0(0) \gg |J_1(\omega_0)| \cong J_2(2\omega_0)$) are easily predicted from eq 6 to be 1.5 and 0.0, respectively. The intriguing prediction in the homonuclear case is the complete disappearance of the observed transition in the slow motion limit. Observation of either extreme value will, of course, only signify whether the motion is "slow" or "fast" in conjunction with Figure 1. Observation of an intermediate value can be directly translated into a *quantitative* measure of mobility. However, it is of paramount importance to note that this straightforward approach presumes isotropic motion (and of course only intradipolar relaxation).

As mentioned previously, the simplest extension of this picture assumes motion characterized by two diffusional constants. Figure 2 is a topographical (contour) plot of the normalized fractional line intensity as a function of overall rotation ($\omega_0\tau_c$) and internal flexibility ($\omega_0\tau_R$). Throughout this paper, we shall

arbitrarily identify $\tau_R \equiv D_{int}^{-1}$ (or, alternatively, the interpretation $\tau_R \equiv (D_{\parallel} - D_{\perp})^{-1}$).

As seen in eq 7, η will depend explicitly upon the angle β; three illustrative values of this angle are presented in Figure 2. The angle $\beta = 54°$ was chosen to illustrate the particular behavior resulting as β approaches the nmr magic angle ($\beta_0 = \cos^{-1} (3^{-1/2}) \cong 54.7°$). The plot for $\beta = 36°$ typlifies any plot for $20° \lesssim \beta \lesssim 45°$ and the plot for $\beta = 90°$ typlifies the range $60° \lesssim \beta \lesssim 90°$. Notice that only subtle differences exist between these two angular ranges. Furthermore, for $0° \leq \beta \lesssim 20°$, there is very little deviation from Figure 1 for *any rate* of internal rotation (for $\beta = 0°$ there is no dependence). For all rotation angles (save those approaching the magic angle), extremely rapid internal rotation results in the fact that the contours of constant $1 + \eta$ approach the limiting values obtained in the absence of internal (or asymmetric) motion (compare asymptotic values with those seen in Figure 1). This fact is true *regardless* of the magnitude of overall motion. This rather surprising result is to be contrasted with the results obtained in ref 6. At intermediate rates, the description becomes much more complicated due to the identity struggle between the variables ω_0, $D(\tau_c)$, and $D_{int}(\tau_R)$, as revealed by a rather lengthy perusal of eq 7. Figure 3 facilitates this analysis. This figure plots contours of the difference values ($\Delta_{rot}\eta$) of NOE enhancements. These difference values are obtained by first calculating η assuming internal rotation (of the magnitude indicated by the vertical axis) and then subtracting the value calculated by assuming no internal rotation. This quantity is then computed as a function of the overall isotropic mobility plotted as the horizontal independent variable. Therefore, this nonnegative quantity is of the simple form

$$\Delta_{rot}\eta = \eta_{D,Dint} - \eta_{D,Dint=0} \qquad (9)$$

Figure 3 assumes that $\beta = 90°$, but, as mentioned earlier, this will give the qualitative behavior of a large range of internal geometries. The full meaning of Figures 1, 2, and 3 is now developed.

If one naively interprets an Overhauser enhancement in terms of Figure 1, how will it differ from a more exact interpretation using Figure 2? Figure 1 shows that between the limits $0.2 < \eta + 1 < 1.3$ (outside of these limits, the NOE experiment loses its usefulness in the present context), $\Delta\eta/\Delta \log (\omega_0\tau_c) \cong -2$. Hence, the *maximum* error introduced into interpretation of τ_c by means of graph 1, would occur when $\omega_0\tau_R \cong 10^{-0.3}$. ($D_{int}/\omega_0 \cong 2$) and $\omega_0\tau_c \cong 10^{-0.5}(D/\omega_0 \cong 1/2)$. As seen in Figure 3, $\Delta_{rot}\eta$ would be on the order of 0.6 resulting in a misinterpretation of τ_c by a factor of 2 ($\cong 10^{0.3}$). Any other pair of diffusional constants would result in a smaller value of $\Delta_{rot}\eta$, hence a smaller misinterpretation of τ_c. Therefore, except for a rather limited range of unique rotational geometries, interpretation of NOE enhancements is quite straightforward, *yielding the overall rotational diffusion constant within a factor of 2 or less*.

Finally, these plots suggest a promising, albeit tedious, range of experiments if one is blessed with the
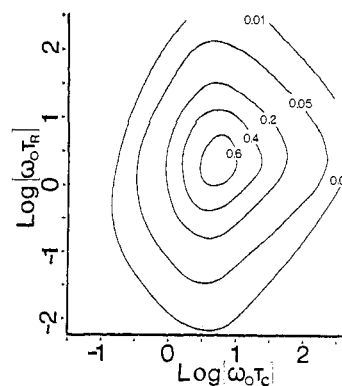

Figure 3. Contour (topographical) plots of the difference values ($\Delta_{rot}\eta$) of the NOE enhancements (calculated assuming internal rotation minus that calculated ignoring internal rotation) as a function of overall isotropic and internal rotation for the given angle of $\beta = 90°$. See eq 9 and related discussion for further details. Contours are for $\Delta_{rot}\eta = 0.01, 0.05, 0.2, 0.4, \text{and} 0.6$.

flexibility of performing NOE experiments a various field strengths. This could, in favorable circumstances, yield not only the overall rotational rate but also the rate of internal rotation. Indeed the motional information obtainable from the NOE experiment, especially when used in conjunction with other studies, appears to be a promising avenue of study.

Conclusion

It has been demonstrated that (homonuclear) NOE enhancement factors which measure $\eta = (J_0(0)/3 - J_2(2\omega_0))/(-J_0(0)/3 + J_1(\omega_0) - 2J_2(2\omega_0))$ yield unique motional information, which for many parameterizations is sensitive to overall isotropic motion of the spin pair.

This information may be combined with T_1/T_2 ratios

$$T_1/T_2 = (-3J_0(0) + 5J_1(\omega_0) - 2J_2(2\omega_0))/(2J_1(\omega_0) - 8J_2(2\omega_0))$$

or $T_2(\omega_0')/T_2(\omega_0)$ ratios

$$T_2(\omega_0')/T_2(\omega_0) = (-3J_0(0) + 5J_1(\omega_0) - 2J_2(2\omega_0))/(-3J_0(0) + 5J_1(\omega_0') - 2J_2(2\omega_0'))$$

As each measurement depends upon different combinations of spectral densities, the experiments provide complementary information about molecular motion.

Also demonstrated is the fact that heteronuclear NOE, which is often sensitive to internal motion, yields very marked differences from those presented in this paper.