DIFFUSION OF OXYGEN IN WATER AND HYDROCARBONS USING AN ELECTRON SPIN RESONANCE SPIN-LABEL TECHNIQUE

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ABSTRACT   The Smoluchowski equation for the bimolecular collision rate of dissolved oxygen molecules with spin labels yielded values for the diffusion constant of oxygen in water that are in agreement with the Stokes-Einstein equation ($D \propto T/\eta$, where η is the macroscopic viscosity) and with published values obtained by conventional methods. Heisenberg exchange at an interaction distance of 4.5 Å occurs with a probability close to one for each encounter. In mixed hydrocarbons (olive oil, paraffin oils) and sec-butyl benzene, $D \propto (T/\eta)^p$, where p lies between 0.5 and 1. Oxygen diffuses in the hydrocarbons between 10 and 100 times more rapidly than predicted from the macroscopic viscosity. Similar results would be expected for diffusion of oxygen in model and biological membranes. Parallel measurements of rotational diffusion of the spin labels show little correlation with measurements of translational diffusion of oxygen. Dipolar interactions between spin labels and oxygen appear negligible except in the limit of highest viscosities.

INTRODUCTION

Not many measurements of the diffusivity of dissolved oxygen in fluids have been made. The subject was reviewed by Himmelblau in 1964 (1), but there have been no reviews since then. Windrem and Plachy (2) provide a search of the literature through 1977. The fundamental definition of diffusion is Fick's second law, the differential equation describing diffusion in a concentration gradient. All available data based on Fick's second law for diffusivity of oxygen in water up to 1971 were collected by St.-Denis and Fell (3), and fitted to the equation

$$D(O_2) = 6.92 \times 10^{-10} (T/\eta), \quad (1)$$

a form consistent with the Stokes-Einstein equation. Here η is the macroscopic viscosity in poise. Krieger et al. (4) report a datum for a hydrocarbon, cyclohexane, but we have located no values for diffusivity of dissolved oxygen in long-chain hydrocarbons that might be considered representative models for diffusion of oxygen in lipid bilayers.

Experimental difficulties in measuring the diffusion of dissolved oxygen in fluids are severe. For example, St.-Denis and Fell cite 23 papers using 11 different methods. The scatter of values at 25°C varied from 1.8 to 2.6 × 10^{-5} cm^2/s. The difficulties arise because oxygen has no convenient radioactive isotopes, requiring the detection of the time evolution of the oxygen concentration at a point in space by some less convenient method.

Smoluchowski applied Fick's first law to collisions of molecules and derived the equation that bears his name

$$\omega = 4\pi R[B] \{D(A) + D(B)\}. \quad (2)$$

Here A and B refer to two kinds of molecules dissolved in a continuum solvent, ω is the frequency of collisions that particular A molecules experience with B molecules, and R is an interaction distance of a few Angstroms (Windrem and Plachy use 4.5 Å for nitroxides as A and oxygen as B). This bimolecular collision rate does not depend on the concentration of A, of course. Note that there are no molecular dimensions other than R in Eq. 2. We introduce the notation D_S whenever a diffusion constant is discussed that is defined by the Smoluchowski equation. If the subscript is absent, D is defined by Fick's second law.

Molecular oxygen is paramagnetic, and this property can be used to measure the bimolecular collision rate by one of several techniques. Eq. 2 should then be modified to relate the experimental observable to the actual collision frequency.

$$\omega_{obs} = 4\pi Rp[O_2]\{D(A) + D_S(O_2)\}, \quad (3)$$

where p is the probability that an observable event is recorded when a collision takes place. The product Rp can, in principle, be adjusted to force agreement of $D(O_2)$ and $D_S(O_2)$.

We have recently published a series of papers (5–9) on measurement of oxygen concentration and diffusion using electron spin resonance (ESR) spectroscopy and observing the effects of oxygen collisions on the spectra of nitroxide

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radical spin labels. We have come to call this family of methods spin-label oximetry. Earlier papers include that of Backer et al. (10) and Povich (11). The dominant magnetic interaction is Heisenberg exchange, which alters both T_1 (the nitroxide spin-lattice relaxation time) and T_2 (the transverse relaxation time, which affects the resolution of superhyperfine couplings with methyl protons). A numerical evaluation of Eq. 3, letting $p = 1$, shows that the bimolecular collision rate is comparable with T_1^{-1} and also with the line width (expressed in frequency units). This fortuitous good match makes the spin-label oximetric methods competitive with other oximetric techniques.

Here we report measurements of nitroxide line broadening due to oxygen collisions in several solvents over a wide range of η/T values, holding the concentration of oxygen constant in a particular solvent. Solvents include water, various glycerol-water mixtures, sec-butyl benzene (SBB), olive oil, and heavy and light paraffin oil. The biological rationale for this choice of solvents is that it provides a basis for extrapolation of our experimental methods to model and biological membranes.

In our previous studies we have become conscious of three aspects of the spin-label oximetric methods that require further investigation. (a) Because ESR line-broadening experiments are fast and easy, it would be of interest to compare diffusion constants obtained from Eq. 3 with values obtained by conventional methods with the hypothesis that measurements of bimolecular collision of oxygen with spin-labels should become a more widely used general technique for studying oxygen diffusivity. (b) In membranes, the viscosity can be high. If the diffusion rate of oxygen past the spin label is sufficiently low (i.e., the translational correlation time is sufficiently long), there can, in principle, be not only Heisenberg exchange contributions but also significant dipole-dipole contributions to the line width. It was therefore desirable to assess the relative importance of these two interactions in solvents that can be considered representative models for diffusion of oxygen in membranes. (c) Our ideas of viscosity or fluidity in membranes as determined by rotational diffusion effects on line widths of spin-label spectra were not useful in understanding diffusion of molecular oxygen. Our studies of these three aspects constitute the subject of this report.

METHODS

Hyde and Sarna (12) studied the interaction of paramagnetic metals with spin labels in solution, and their paper provides the methodological background for the present work. See particularly their Fig. 8. At low viscosities where Heisenberg exchange dominates, the incremental contributions to the line widths ΔH_{p-p} decrease as the viscosity η increases. At high viscosities where dipolar contributions dominate, the values of ΔH_{p-p} increase as η increases. A characteristic minimum is therefore observed when line width is plotted vs. η/T. Measurements of ΔH_{p-p} have been plotted in the several solvents over four orders of magnitude in η/T.

The spin-label tanone (also called tempone)
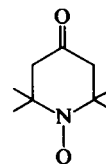
was used at a concentration of 10^{-4} M. Sec-butyl benzene was saturated with 1 atm oxygen (not air) at 30°C, paraffin oils (Matheson, Coleman and Bell, Norwood, OH) at 15°C, olive oil (Sigma Chemical Co., St. Louis, MO) at 15°C, and water and glycerol water at 60°C. The sample tubes were then sealed, ensuring that the concentration of dissolved oxygen remained constant for each sample. The high temperature for preparation of water and glycerol-water samples was necessary to avoid formation of bubbles when making measurements at high temperature. Absolute measurements of oxygen concentrations were not made, but their approximate values may be taken from the literature, Table I.

Bimolecular collision rates were related to changes in peak-to-peak line widths by the equation

$$\Delta\omega_{obs} = \gamma\Delta H_{p-p}\frac{\sqrt{3}}{2}, \qquad (4)$$

where γ is the magnetogyric ratio of the electron. This equation is appropriate in principle if the line shape in the absence of oxygen is Lorentzian. No specific measurements of line shapes were made and no more elaborate deconvolution procedures used. For more accurate measurements such a procedure would be appropriate.

Correlation times were obtained using the expression

$$\tau_{2B} = 6.51 \, \Delta H \left[\left(\frac{h_0}{h_{-1}}\right)^{1/2} - \left(\frac{h_0}{h_{+1}}\right)^{1/2}\right] \times 10^{-10}s, \quad (5)$$

where $h_{+1,0,-1}$ refer to the heights of the three spin-label lines (17). Tanone was used both for oxygen broadening experiments and measurements of rotational correlation times. Samples were saturated with 1 atm nitrogen for these measurements (at 60°C for glycerol water, 15°C for olive oil, 30°C for SBB, and 15°C for paraffin oils).

ESR measurements were made with a spectrometer (E9; Varian Associates, Palo Alto, CA) in normal configuration. The microwave power was 1 mW, and the modulation amplitude ~5 times less than the peak-to-peak line width. Temperatures were measured by a thermocouple just outside the sample tube and just above the active region of the cavity. Every point was repeated several times.

TABLE I
OXYGEN CONCENTRATION DATA

Sample	T°C	[O_2]	Comments
		mM	
Water	60	0.67	Extrapolated from reference 13
Sec-butyl Benzene	30	8.5	Value from reference 14 for toluene at 25°C
Olive oil	15	2.7	Reference 13 data on water and reference 16 data on partition coefficient
Heavy and light paraffin oil	18	5.0	Reference 15

Viscosities for glycerol water and olive oil were taken from reference 18 and for sec-butyl benzene from reference 19. We made measurements for paraffin oil in two ways: by measuring the time for a steel ball to fall and for oil to flow through Canon-Fenske capillaries. We obtain at 4, 10, 20, 37, and 60°C, values of 223, 176, 110, 54, and 29 cP and 156, 96, 64, 24, and 13 cP for heavy and light paraffin oil, respectively.

RESULTS AND DISCUSSION

Fig. 1 shows a plot of $\Delta\omega(p\text{-}p)$ vs. T/η for water. The data fall on a straight line as predicted by the Stokes-Einstein equation, just as was found by St.-Denis and Fell (3) using data based on measurements of diffusion of oxygen in a concentration gradient.

If we assume a value for Rp, Eq. 3, of 4.5 Å

$$D_s(O_2) = 4.76 \times 10^{-10} (T/\eta). \tag{6}$$

The coefficient is in remarkable agreement with that of St.-Denis and Fell (3). Perhaps the most significant aspect of the close agreement between Eqs. 1 and 6 is that p, the probability that a Heisenberg exchange event occurs when a collision occurs, must be close to unity. Molin et al. (20) and Salikhov et al. (21) have pointed out that if the translational correlation time, τ_c, is sufficiently short, p will decrease as $T_{1(O_2)}\tau_c/(1 + KT_{1[O_2]}\tau_c$, where K depends on the exchange integral. Apparently $KT_{1(O_2)}\tau_c \gg 1$ for oxygen molecules colliding with tanone molecules even for viscosities as low as 1 cP. All other solvents studied here have viscosities greater than water.

We can further infer from Fig. 1 that nonsecular dipole-dipole contributions to the line widths are negligible. These contributions to T_1 (and therefore to T_2) will depend on the product of the microwave frequency and τ_c, and will not have the simple T/η dependence of Fig. 1, as noted by Hyde and Sarna (12). Throughout this paper, we make the assumption that $p \simeq 1$, and therefore $\omega_{obs} = \omega$, the justification being the agreement between Eqs. 1 and 6.

Hyde and Sarna (12) varied η/T of glycerol-water

FIGURE 2 Line width difference in field units of the center ($m_1 = 0$) line of tanone in water and water glycerol samples saturated with 1 atm molecular oxygen at 60°C and samples saturated with 1 atm molecular nitrogen at 60°C as a function of η/T. Also shown is the line width difference data for olive oil saturated with O_2 and with N_2 at 15°C. Note the different ordinate scales.

mixtures by four orders of magnitude by a combination of temperature and composition changes, linking the various segments. Such an approach assumes that glycerol-water mixtures are like high viscosity water with other properties unchanged. This is obviously a rough approximation but can, nevertheless, give insight into transport properties of water. We followed this procedure to obtain the oxygen broadening data of Fig. 2 and the rotational correlation time data of Fig. 3. Superimposed on these two figures are comparable data for olive oil. Because Battino et al.'s work (16) and our own (9) demonstrate that the solubility of oxygen is four times higher in olive oil than in water, the ordinate on the right side of Fig. 2 has been compressed by a factor of four. Thus, in accordance with Eqs. 2 and 3, Fig. 2 permits the direct comparison of diffusion of oxygen in water and olive oil. (Diffusion of the spin label is considered negligible compared with diffusion of O_2).

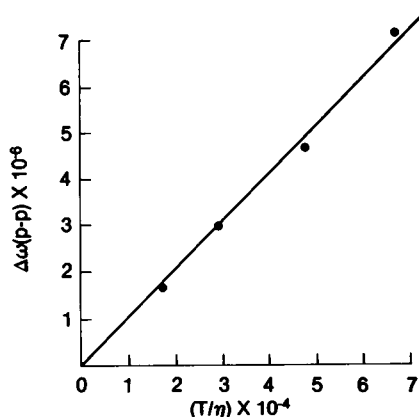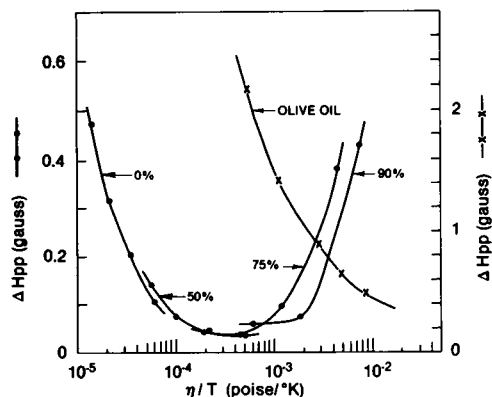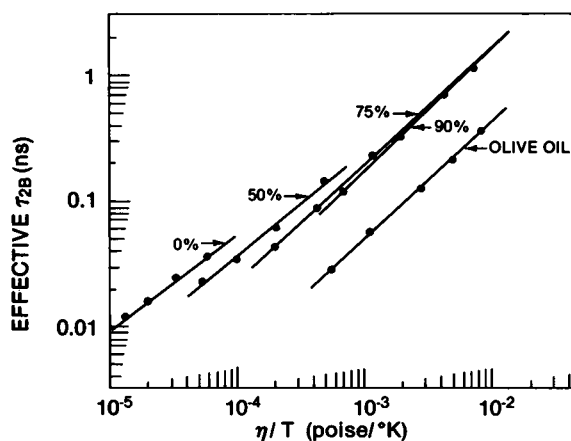The segments for the various glycerol-water mixtures fit

FIGURE 1 Line width difference in frequency units of the center ($m_1 = 0$) line of tanone in water between a sample saturated with 1 atm molecular oxygen at 60° and a sample saturated with 1 atm molecular nitrogen at 60°C as a function of T/η (°K/P).

FIGURE 3 Rotational correlation times of tanone in water, water-glycerol mixtures, and olive oil as a function of η/T ($P/°K$). See Eq. 5.

fairly well in Fig. 2. A minimum in line width is observed, which is consistent with dominant Heisenberg exchange contributions to line width at low viscosities and dominant secular dipolar contributions at high temperature. We interpret the olive oil data of Fig. 2 as being simply displaced by two orders of magnitude along the abscissa from the glycerol-water data. That is, there is about a factor of 100 difference between the microscopic viscosities that control translational diffusion of molecular oxygen and the macroscopic viscosities. Oxygen diffuses through olive oil about as easily as through water. This conclusion also follows from the work of Windrem and Plachy (2), Subczynski and Hyde (7), and Kusumi et al. (8). We expect a marked difference in transport properties in displays such as Fig. 2 when the ratio of solute-to-solvent molecular weights is much less than 1.

There is an implicit assumption, here, that Rp is the same in olive oil as in water. The assumption that exchange is determined by properties of the colliding molecules rather than by solvent molecules is commonly made. It would nevertheless be appropriate in future work to measure the product by comparing $D(O_2)$ and $D_S(O_2)$ in various solvents.

Fig. 3 shows rotational diffusion data for the spin label. Although the slopes are all about one, as predicted by the Stokes-Einstein equation for rotational diffusion

$$\tau_2 = \frac{4}{3} \pi r^3 \frac{\eta}{kT}, \tag{7}$$

they are in fact somewhat <1 (0.72 for water; 0.75 [50%], 0.91 [75%], and 0.97 [90%] for the glycerol-water mixtures; and 0.93 for olive oil). Eq. 7 predicts that all data in Fig. 3 should fall on the same 45° line. Clearly the glycerol water and olive oil data are displaced with respect to each other by about an order of magnitude in η/T. The combined observations of altered slope and displacement indicate that a microviscosity concept must be invoked to describe rotational diffusion of tanone, and we note that there appears to be little connection between translational diffusion of molecular oxygen and rotational diffusion of the spin label. Fig. 4 shows the bimolecular collision rate of molecular oxygen with tanone, using only the lower viscosity data that are dominated by Heisenberg exchange interactions. In contrast to the slope of 1.0 shown in Fig. 1, slopes are 0.54 (olive oil), 0.72 (SBB), 0.62 (light paraffin oil), and 0.86 (heavy paraffin oil).

Evans et al. (22, 23) have advocated an empirical expression

$$D\eta^p = \frac{AT}{298.2K} \tag{8}$$

to describe translational diffusion of small molecules. We would prefer a form

$$D(\eta/T)^p = A', \tag{9}$$

which appears to be equally consistent with the data. The
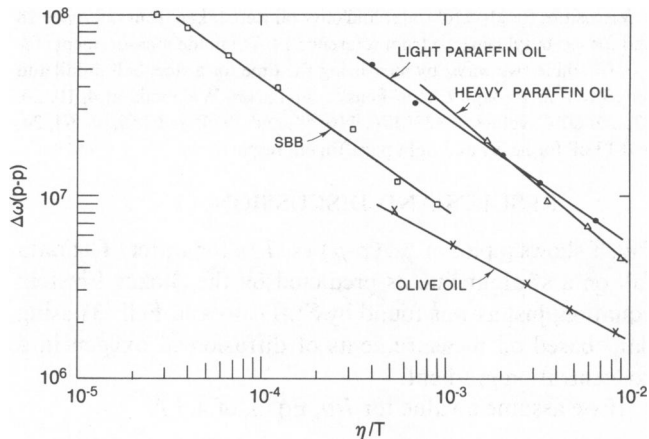

FIGURE 4 Line width difference data in frequency units of the center $(m_I - 0)$ line of tanone in sec-butyl benzene, light paraffin oil, heavy paraffin oil, and olive oil (see text for sample preparation) as a function of η/T ($P/°K$).

range of variation of p reported by Evans et al. (who used a radioactive tracer technique based on Fick's second law) is similar to that found here using the Smoluchowski equation. However, these authors indicate that over a certain range of solvents, p and A are properties of the solute, whereas our data indicate that they are properties of both solute and solvent. Like Evans and his colleagues, we are impressed by the linearity shown in plots such as Fig. 4, and feel that a theoretical explanation ought to be possible. Povich (24) reports a quite different dependence in silicon fluids.

Fig. 5 shows line width data for SBB and the two paraffin oils over a wide range of η/T in a semilog display similar to that of Fig. 2. The scales of the abscissas have been adjusted by the concentration of dissolved oxygen. To a fair approximation, all data could be superimposed on a universal curve simply by assigning a microviscosity that is
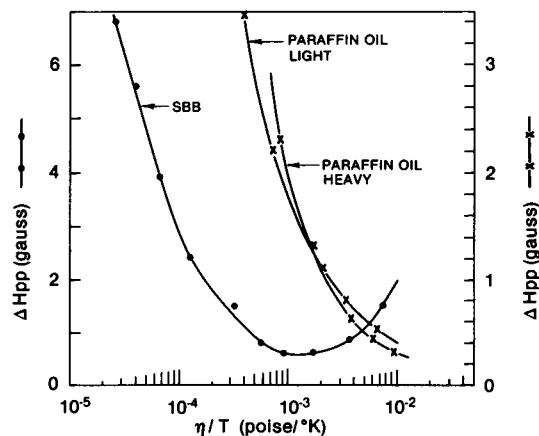

FIGURE 5 Line width difference data in field units of the center $(m_I - 0)$ line of tanone in sec-butyl benzene, light paraffin oil, heavy paraffin oil (see text for sample preparation) as a function of η/T. Note the different ordinate scales.
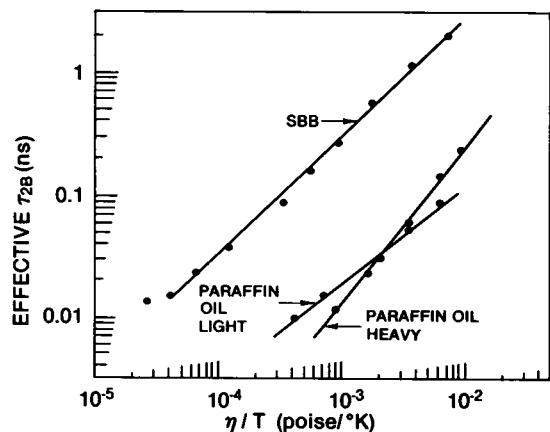
FIGURE 6 Rotational correlation times of tanone in sec-butyl benzene, light paraffin oil, and heavy paraffin oil as a function of η/T. See Eq. 5

proportional to the viscosity with a constant of proportionality that is a property of the solvent.

At high viscosities in 90% glycerol and SBB, the line widths increase with viscosity. This is a manifestation of dipolar interactions between oxygen and spin label. At the limit of infinitely high viscosity, the Van Vleck method of moments (25) could be used to determine the static broadening from the A component of the dipolar Hamiltonian, and the moment approach of Hyde and Rao (26) could be used to determine contributions from the B, C, D, E, and F components. More complete analysis would be necessary to establish the magnitudes of the contributions of these various terms. Because nothing is known about the spin-lattice relaxation time of oxygen, analysis is complicated. In any event, all terms in the dipolar Hamiltonian will lead to a linear dependence in concentration with the distance of closest possible approach of O_2 and spin label being a key parameter.

Data for rotational diffusion of tanone in SBB and the paraffin oils are shown in Fig. 6. Note the difference in slopes between the two oils. Comparing Fig. 3, all water, glycerol water, and SBB data fall approximately on the same 45° line indicating that Eq. 7 is fairly good when the size of the solvent molecule is similar to or larger than the solute molecule. The oils, however, present a much more complex behavior.

CONCLUSIONS

Our hypothesis that the Smoluchowski equation, Eq. 3 with $R = 4.5$ Å and $p = 1$, can be used to describe bimolecular encounters between dissolved molecular oxygen and spin labels over all ordinary solvents and conditions, has survived the tests of the present paper and our previous papers and is now considered reasonably well-established.

Using this approach, translational diffusion of oxygen can be measured more readily and more accurately in a wider range of solvents than was previously possible. In long-chain hydrocarbons, oxygen diffusion is about as facile as in water at the same temperature, even though there may be one or two orders of magnitude difference in macroscopic viscosities. We would anticipate a similar conclusion for diffusion of oxygen in model and biological membranes.

Rotational diffusion of spin labels and translational diffusion of molecular oxygen are separate subjects not easily related to each other or to macroscopic solvent properties.

Dipolar contributions of molecular oxygen to the line width of spin labels appears generally subordinate to Heisenberg exchange contributions. The data indicate that the exchange integral between molecular oxygen and spin label is sufficiently large that all encounters are of a strong-exchange type.

REFERENCES

1. Himmelblau, D. M. 1964. Diffusion of dissolved gases in liquids. Chem. Rev. 64:527–550.
2. Windrem, D. A., and W. Z. Plachy. 1980. The diffusion-solubility of oxygen in lipid bilayers. Biochim. Biophys. Acta. 600:655–665.
3. St.-Denis, C. E., and C. J. D. Fell. 1971. Diffusivity of oxygen in water. Can. J. Chem. Eng. 49:885.
4. Krieger, I. M., G. W. Mulholland, and C. S. Dickey. 1967. Diffusion coefficients for gases in liquids from the rates of solution of small gas bubbles. J. Phys. Chem. 71:1123–1129.
5. Lai, C.-S., L. E. Hopwood, J. S. Hyde, and S. Lukiewicz. 1980. ESR studies of O_2 uptake by Chinese hamster ovary cells during the cell cycle. Proc. Natl. Acad. Sci. USA. 79:1166–1170.
6. Popp, C. A., and J. S. Hyde. 1981. Effects of oxygen on EPR spectra of nitroxide spin-label probes of model membranes. J. Magn. Reson. 43:249–258.
7. Subczynski, W. K., and J. S. Hyde. 1981. The diffusion-concentration product of oxygen in lipid bilayers using the spin-label T_1 method. Biochim. Biophys. Acta. 643:283–291.
8. Kusumi, A., W. K. Subczynski, and J. S. Hyde. 1981. An oxygen transport parameter in membranes as deduced by saturation recovery measurements of spin-lattice relaxation time of spin labels. Proc. Natl. Acad. Sci. USA. 79:1854–1858.
9. Subczynski, W. K., and J. S. Hyde. 1983. Concentration of oxygen in lipid bilayers using a spin-label method. Biophys. J. 41:283–286.
10. Backer, J. M., V. G. Budker, S. I. Eremenko, and Yu. N. Molin. 1977. Detection of the kinetics of biochemical reactions with oxygen using exchange broadening in the ESR spectra of nitroxide radicals. Biochim. Biophys. Acta. 460:152–156.
11. Povich, M. J. 1975. Measurement of dissolved oxygen concentrations and diffusion coefficients by electron spin resonance. Anal. Chem. 47:346–347.
12. Hyde, J. S., and T. Sarna. 1978. Magnetic interactions between nitroxide free radicals and lanthanides or Cu^{2+} in liquids. J. Chem. Phys. 68:4439–4447.
13. Hitchman, M. L. 1978. Measurement of Dissolved Oxygen. John Wiley and Sons, Inc., New York. 255.
14. Wilhelm, E., and R. Battino. 1973. Thermodynamic functions of the solubilities of gases in liquids at 25°C. Chem. Rev. 73:1–9.
15. Linke, W. F. 1965. Solubilities. Inorganic and Metal Organic

Compounds II. Amer. Chem. Soc., Washington, DC. 4th edition. 1233–1236.

16. Battino, R., F. D. Evans, and W. F. Danforth. 1968. Solubilities of seven gases in olive oil with reference to theories of transport through the cell membrane. *J. Am. Oil Chem. Soc.* 45:830–833.

17. Stone, T. J., T. Buckman, P. L. Nordio, and H. M. McConnell. 1965. Spin-labeled bimolecules. *Proc. Natl. Acad. Sci. USA.* 54:1010–1017.

18. Weast, R. C. Handbook of Chemistry and Physics, 55th Edition. Chemical Rubber Co., Cleveland, OH. D-205, F-54.

19. Barlow, A. J., J. Lamb, and A. J. Matheson. 1966. Viscous behavior of supercooled liquids. *Proc. Roy. Soc. (Lond.).* 292:322–342.

20. Molin, Yu. N., K. M. Salikhov, and K. I. Zamaraev. 1980. Spin Exchange. Springer-Verlag, Berlin. 242.

21. Salikhov, K. M., A. B. Doctorov, Yu. N. Molin, and K. I. Zamaraev.

1971. Exchange broadening of ESR lines for solutions of free radicals and transition metal complexes. *J. Magn. Reson.* 5:189–205.

22. Evans, D. F., T. Tominaga, and C. Chan. 1979. Diffusion of symmetrical and spherical solutes in protic, aprotic, and hydrocarbon solvents. *J. Solution Chem.* 8:461–478.

23. Evans, D. F., T. Tominaga, and H. T. Davis. 1981. Tracer diffusion in liquids. *J. Chem. Phys.* 74:1298–1305.

24. Povich, M. J. 1975. Electron spin resonance oxygen broadening. *J. Phys. Chem.* 79:1106–1109.

25. Van Vleck, J. H. 1948. The dipolar broadening of magnetic resonance lines in crystals. *Phys. Rev.* 74:1168–1183.

26. Hyde, J. S., and K. V. S. Rao. 1978. Dipolar induced electron spin-lattice relaxation in unordered solids. *J. Magn. Reson.* 29:509–516.