Locating Spin Traps in Heterogeneous Media by ^{13}C NMR Spectroscopy. Investigations in SDS Micelles, DMPC Vesicles, and Rat Liver Microsomes†

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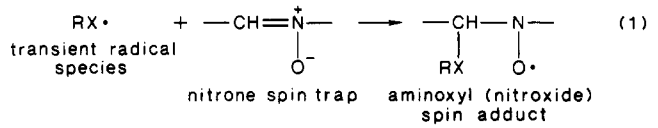
Complete ^1H and ^{13}C NMR assignments of the chemical shifts of α-phenyl-*N*-*tert*-butylnitrone (PBN), α-(4-(dodecyloxy)phenyl)-*N*-*tert*-butylnitrone (4-DOPBN), and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) are reported in D_2O and $CDCl_3$; sodium 5-carboxy-5-methyl-1-pyrroline *N*-oxide (SCMPO) is reported in D_2O. The effect of solvent polarity on their chemical shifts was studied in a large number of solvents. Of all the ^{13}C resonances of these spin traps, the nitronyl ^{13}C chemical shift exhibited by far the largest solvent shift (ca. 15–20 ppm from nonpolar organic to aqueous solutions), which is approximately linear with solvent polarity parameters such as Reichart's $E_{T(30)}$ or Kosower's *Z* values. From these correlations the micropolarities experienced by the various nitrones, as indicated by their ^{13}C-nitronyl chemical shifts, were gauged in the heterogeneous systems sodium dodecyl sulfate (SDS) micelles, dimyristoyl phosphatidylcholine vesicles, and rat liver microsomal preparations. The dual nature of nitrone spin traps as probes for free-radical events and as markers for solvent micropolarity provides a means of investigating the location of spin traps in heterogeneous media such as the SDS and DMPC model-membrane assemblies and in related biological systems such as rat liver microsomal preparations.

Introduction

Impetus for the work described here stems from an interest in the use of nitrones to study free-radical phenomena[1] in vitro,[2] in vivo,[2] as well as in live cellular and component systems.[3] As a model for these heterogeneous systems, we have recently reported on studies with aqueous sodium dodecyl sulfate (SDS) solutions and speculated upon the residency of various hydrophilic and hydrophobic nitrones within these micelles.[4] The NMR method presented herein for the first time correlates the ^1H and ^{13}C chemical shifts of four nitrone spin traps with solvent polarity as indicated by several standard polarity parameters. From these correlation results it is possible to use the ^1H and ^{13}C chemical shifts of nitrones to gauge the polarity of the microenvironment experienced by the nitrone spin trap in heterogeneous systems. We report on our work with sodium dodecyl sulfate (SDS) micelles, dimyristoyl phosphatidylcholine (DMPC) vesicles, and rat liver microsomal (RLM) preparations. Knowledge of the micropolarities surrounding the individual nitrones may provide insight about the location of radical processes in a biological milieu[1-3] since nitrone spin traps can intercept transient radicals in solution under biologically relevant conditions to produce persistent aminoxyl spin adducts whose electron paramagnetic resonance (EPR) parameters can identify the trapped radical.



Since the nitrogen hyperfine splittings (HFS's) of most aminoxyl spin adducts[6] and spin labels[7] increase approximately linearly with increases in solvent polarity (e.g. $E_{T(30)}$[8]), the location of these species in model-membrane systems[4,5,9] is readily estimated by EPR spectroscopy. Additional information on the locations of aminoxyls

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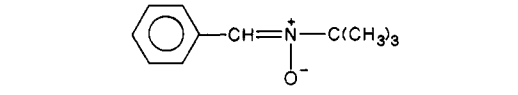
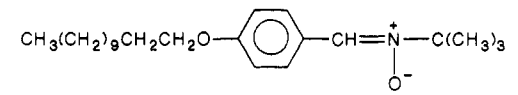
within micelles,[10a] inverse micelles,[10b] and frozen micelles[10c] has recently been obtained by using advanced EPR techniques such as ENDOR (electron nuclear double resonance)[10a,b] and electron spin echo envelope modulation.[10c]

In contrast, attempts to ascertain the solubilization sites of nitrone spin traps in micellar systems (e.g. by UV spectroscopy[5e]) have been unsuccessful.[11] Thus we decided to investigate the solvent sensitivity of the nitronyl NMR chemical shifts to establish a scale of chemical shift vs solvent polarity for use in heterogeneous systems of unknown polarity.

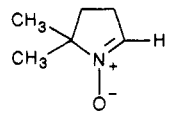
The nitrones used in this study are α-phenyl-*N*-*tert*-butylnitrone (PBN), α-(4-(dodecyloxy)phenyl)-*N*-*tert*-butylnitrone (4-DOPBN), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), and sodium 5-carboxy-5-methyl-1-pyrroline *N*-oxide (SCMPO).



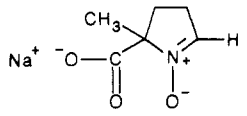
PBN



4-DOPBN



DMPO



SCMPO

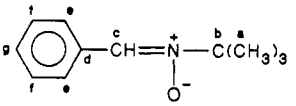
During this study we found that both the nitronyl ^1H and ^{13}C chemical shifts exhibited increases with increase in solvent polarity. It is noteworthy that the ^{13}C NMR chemical shifts of certain other solubilizates (e.g. carbonyl) can be sensitive to solvent polarity[12] and that this feature has been employed to probe micelle structure[13a,b] and water content of phospholipid bilayers.[13c]

Experimental Section

The aromatic nitrones, PBN,[14a] PBN-*nitronyl*-^{13}C[14a] (synthesized from benzaldehyde-*carbonyl*-^{13}C, Merck, Sharpe, and Dohme), 4-DOPBN,[4a] as well as the aliphatic cyclic nitrones DMPO[14b] and SCMPO[14b] were all synthesized by recently reported methods. PBN and DMPO are also available commercially (from Sigma or Aldrich). The deuterated solvents were purchased from Merck, Sharpe, and Dohme. The SDS was electrophoresis grade (from Bio-Rad).

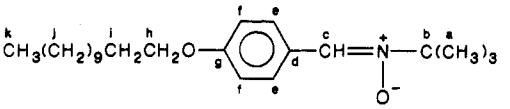
Large unilamellar vesicles (LUV's) composed of the phospholipid dimyristoyl phosphatidylcholine (DMPC) were prepared by employing an extrusion device similar to that available from Lipex Biomembranes, Inc., Vancouver, B.C. The method was similar to that described in the literature.[15a,b] Briefly, dry >99% pure DMPC (from Sigma) was vortexed in 0.05 M phosphate buffer, pH 7.4. The multilamellar vesicles (MLV's) produced were extruded at least 10 times through two stacked polycarbonate filters, pore size 100 nm (Nucleopore Corp., Pleasanton, CA.) employing pressures of up to 800 lb/in.^{2} of nitrogen gas from a

Table I. ^1H and ^{13}C NMR Assignments for PBN



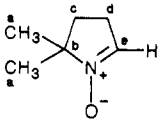
position	^1H NMR		^{13}C NMR	
	$CDCl_3$	D_2O	$CDCl_3$	D_2O
a	1.62	1.57	28.30	29.55
b			70.77	73.47
c	7.56	8.00	129.69	139.91
d			130.84	131.35
e	8.29–8.33	8.24–8.26	128.59	131.96
f	7.38–7.44	7.52–7.58	128.19	132.78
g	7.38–7.44	7.52–7.58	129.88	134.67

Table II. ^1H and ^{13}C NMR Assignments for 4-DOPBN



position	^1H NMR, $CDCl_3$	^{13}C NMR, $CDCl_3$
a	1.57	22.65
b		70.05
c	7.42	130.83
d		123.69
e	8.24	130.92
f	6.88	114.35
g		160.70
h	3.98	68.14
i	1.20–1.46	31.90
j	1.20–1.46	28.30–29.61
k	0.88	14.08

Table III. ^1H and ^{13}C NMR Assignments for DMPO



position	^1H NMR		^{13}C NMR	
	$CDCl_3$	D_2O	$CDCl_3$	D_2O
a	1.43	1.41	24.37	26.16
b			25.31	26.95
c	2.14	2.18	34.07	35.13
d	2.59	2.71	73.55	76.39
e	6.80	7.19	132.35	145.20

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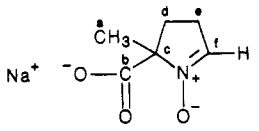
Table IV. ^1H and ^{13}C NMR Assignments for SCMPO



position	^1H NMR, D_2O	^{13}C NMR, D_2O
a	1.59	28.90
b		177.25
c		83.78
d	2.04–2.11	23.87
e	2.50	35.65
f	7.10	147.34

N_2 cylinder. Extrusion was conducted at 30–35 °C above the DMPC phase-transition temperature. Aliquots of the vesicle solutions were examined by quasi-elastic light scattering and freeze-fracture electron microscopy. Quasi-elastic light scattering revealed that vesicle diameters were in a narrow range of 70–134 nm, with a mean diameter of 98 nm. Freeze-fracture electron microscopy showed that the extruded vesicles were very uniform in size, centered around 100 nm in diameter. They were spherical and unilamellar, as indicated by an absence of cross fractures.[15b]

The rat liver microsomal (RLM) preparations (in 150 mM phosphate buffer, pH 7.4) were isolated according to published procedures[2b] and contained approximately 40 mg/mL of protein.

The NMR spectra in the various solvents and SDS micelles were recorded at 22 °C on either a Bruker AM 300 (Milton) or a Bruker WP 400 (Guelph) spectrometer. The DMPC vesicle solutions were studied at 30 °C because the phase-transition temperature for DMPC is close to 22 °C.[15c] The chemical shifts are accurate to within 0.1 ppm. The NMR spectra were generally recorded while locked to the deuterium signals of the respective solvents. The chemical shifts were measured relative to tetramethylsilane, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (external standards), or the known chemical shifts of the neat solvents (internal standards). The pure solvent studies were performed with 0.05 M (or more dilute) solutions of the nitrones. The ratio of SDS to nitrone and DMPC to the nitrone was 25 to 1 or greater. The nitrone concentration was 0.6 mM (or lower) for the RLM's. Typical spectrometer settings (Bruker AM 300) for the ^{13}C NMR spectra (e.g. PBN-*nitronyl*-^{13}C) in the DMPC or RLM systems were as follows: sweep width = 16 000 Hz, number of scans = 2500, pulse width = 2.0 μs (using a 20° flip angle, Hz/Pt = 1, acquisition time = 1.049 s. Unambiguous assignment of some of the nitronyl and other ^{13}C NMR peaks required the use of high-resolution NMR spectroscopy and the application of 2D NMR ^1H–^{13}C correlation sequences[16] and ^{13}C labeling[17] to clarify complex spectral overlap (Tables I–IV).

The EPR spectrum of di-*tert*-butylaminoxyl in 5 M aqueous LiBr was recorded on a Bruker ER 200D EPR spectrometer operating in the standard X-Band mode. The purpose of this experiment was to estimate independently (i.e. not by the solvent sensitive nitronyl NMR chemical shifts) the solvent polarity parameter $E_{T(30)}$[8] for a highly ionic aqueous medium (i.e. 5 M LiBr).

The hydrophobicity data (i.e. octanol/water partition coefficients) for the nitrones, PBN, 4-DOPBN, and DMPO were determined by GC techniques.[18] These data were collected isothermally (200 °C) with a Hewlett-Packard 5710A chromatograph (FID). The columns were 0.25 in. (i.d.) and 8 ft length (for PBN and DMPO), and 2 ft length (for 4-DOPBN), and packed with CSP 633 (a carbowax-type stationary phase).

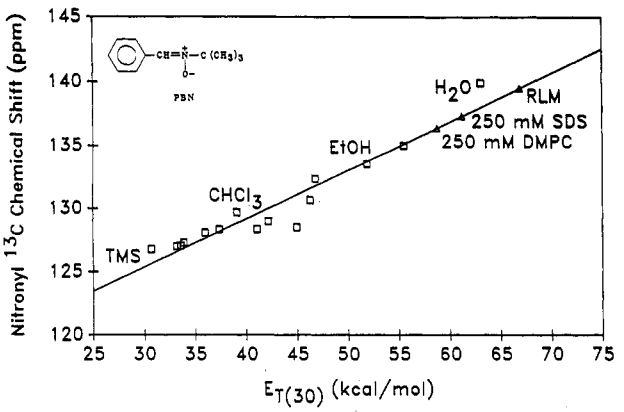


Figure 1. Plot of the nitronyl ^{13}C NMR chemical shifts of PBN vs the solvent polarity parameter $E_{T(30)}$.[8a] Points with $E_{T(30)}$ values estimated from the regression line (i.e. 250 mM SDS, 250 mM DMPC and RLM) are indicated by triangles.

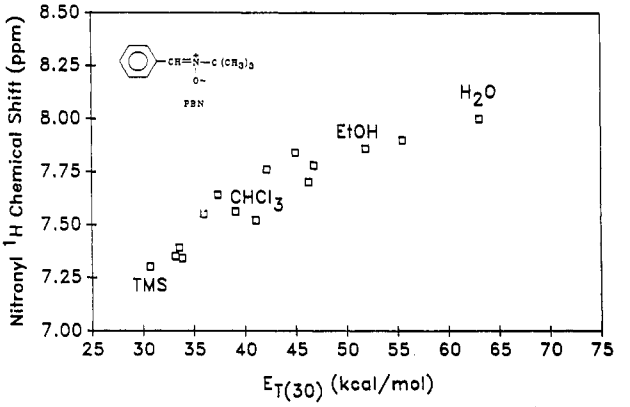
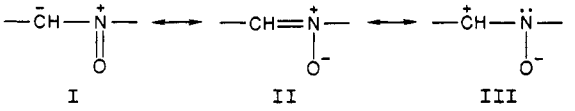


Figure 2. Plot of the nitronyl ^1H NMR chemical shifts of PBN vs the solvent polarity parameter ($E_{T(30)}$).[8a]

Results and Discussion

Since it is known that a polar function with an electron-deficient carbon nucleus generally causes an increase in the ^{13}C NMR chemical shift,[19] a possible solvent sensitivity for the nitronyl ^{13}C chemical shift could be expected; i.e. the resonance form with the greatest charge separation (i.e. III) should be better stabilized in polar solvents than the other two canonical forms (i.e. I and II).



I II III

The ^1H and ^{13}C NMR spectra of PBN in 16 pure solvents ranging from tetramethylsilane (TMS) to water were obtained. It was found that the nitronyl ^{13}C chemical shift showed the greatest sensitivity to solvent polarity. As an illustration, the chemical shifts of all PBN proton and carbon positions were compared in the solvents $CHCl_3$ and D_2O (Table I). The nitronyl (position C) ^{13}C chemical shift showed the most dramatic change.

The ^{13}C nitronyl chemical shift of PBN vs solvent polarity $E_{T(30)}$ gives a plot (Figure 1) with a reasonably good

(16) Maudley, A. A.; Ernst, R. R. *Chem. Phys. Lett.* **1977**, *50*, 368–372. In addition nitronyl ^{13}C-labeled PBN (cf. Janzen, et al., ref 17 was used to confirm the various peak assignments and to reduce the spectral accumulation times and spectral complexity.

(17) Janzen, E. G.; Oehler, U. M.; Haire, D. L.; Kotake, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6858–6863.

(18) For octanol/water partition coefficients of some related nitrones: (a) e.g. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) and analogues: Turner, M. J. III; Rosen, G. M. *J. Med. Chem.* **1986**, *29*, 2439–2444. (b) e.g. 4-Substituted α-phenyl-*N*-*tert*-butylnitrones (PBN's)): Acree, W. E., Jr.; Bacon, W. E.; Leo, A. *Int. J. Pharm.* **1984**, *20*, 209–211.

(19) For example, see: (a) Yijima, C.; Tsujimoto, T.; Suda, K.; Yamauchi, M. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2165–2170. (b) Nelson, G. L.; Levy, G. C.; Cargioli, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 3089–3094.

(20) Kosower, E. M. *J. Am. Chem. Soc.* **1958**, *80*, 3253–3260. A plot of the nitronyl-^{13}C chemical shift of PBN vs Kosower's *Z* values gave a line with $r^2 = 0.96$. *Z* values (in kcal/mol) derived from the UV spectra of 1-ethyl-4-carbomethoxypyridinium iodide are the following: dichloromethane (64.2), 2-propanone (65.7), dimethyl sulfoxide (65.7), nitromethane (71.1), 2-propanol (76.3), ethanol (79.6), methanol (83.6), water (94.6).

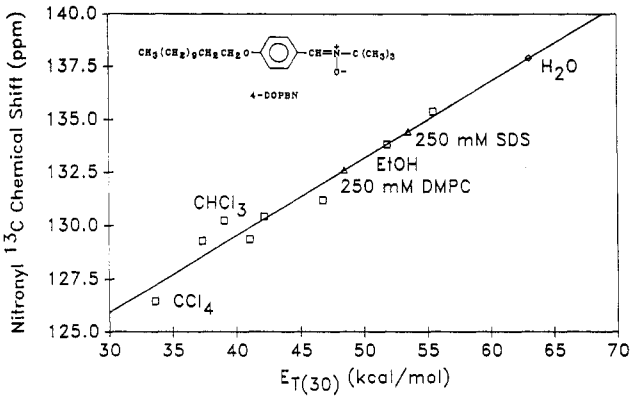


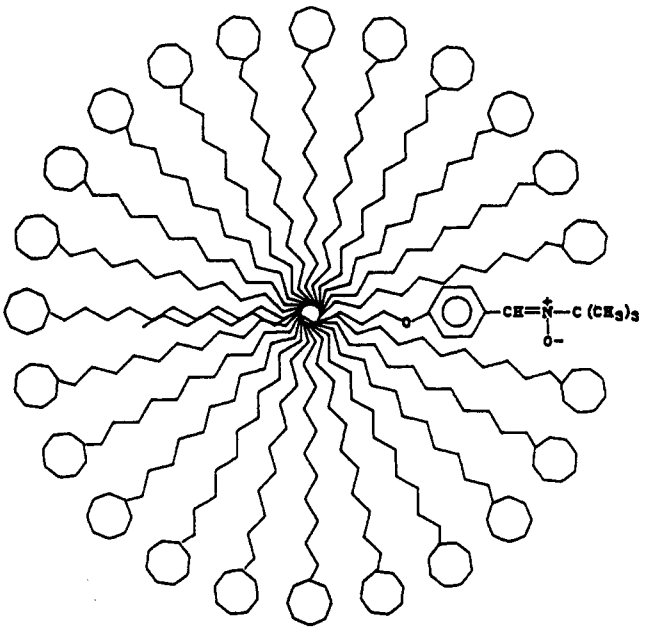
Figure 3. Plot of the nitronyl ^{13}C NMR chemical shifts of 4-DOPBN vs the solvent polarity parameter ($E_{T(30)}$).[8a] The $E_{T(30)}$ values for SDS and DMPC were estimated from the regression line and are indicated by triangles. The nitronyl-^{13}C NMR chemical shift for 4-DOPBN in water was also estimated from the regression line and is indicated by a diamond.

linear correlation (r^2 = 0.92). The ^{13}C chemical shift of nitrones also correlates quite well with other indicators of solvent micropolarity such as Kosower's Z values (UV based), Knauer's a^N values (EPR based),[7,21] as well as Maciel's[12a] and Menger's[13a] carbonyl ^{13}C chemical shift scales (NMR based).[22] A plot of the nitronyl ^{1}H chemical shift vs solvent polarity is more complex (Figure 2). Because the correlation between the ^{13}C chemical shift and solvent polarity appears to be more well behaved, this parameter was selected to gauge the location of the nitrones in the heterogeneous systems. In addition, the chemical shift range (tetramethylsilane to water) is much larger for the ^{13}C plot (13.5 ppm) than that for the corresponding ^{1}H plot (0.7 ppm).

The ^{13}C plot (Figure 3) for the hydrophobic nitrone 4-DOPBN (see Table II) in tetrachloromethane, tetrahydrofuran, trichloromethane, dichloromethane, 2-propanone, 2-propanol, ethanol, and methanol also gave a reasonable good linear correlation (r^2 = 0.92).

Quite clearly the ^{13}C NMR data for PBN and 4-DOPBN (Figures 1 and 3) indicate that the nitronyl functions of these spin traps reside in very polar environments in heterogeneous systems. The nitronyl ^{1}H chemical shift for PBN (Figure 2) is also indicative of a very polar microenvironment for the location of PBN (approximately that of a pure ethanol solution). However PBN and 4-DOPBN are quite hydrophobic. The octanol/water partition coefficients[18] were found to be 15.1 for PBN and $\sim 10^4$ for 4-DOPBN. It is noteworthy that although a nitrone may be represented by resonance structures with dipolar bonds this functional group is neutral overall and is similar in polarity to a ketone group. 4-DOPBN however is so hydrophobic that it is impossible to obtain the ^{13}C NMR spectrum in a purely aqueous system! Thus these aromatic nitrones prefer to be sequestered within the SDS micellar, DMPC vesicular, or equivalent RLM structures[23] rather

Chart I. 4-DOPBN in an SDS Micelle



than dissolved in the bulk aqueous phase. A likely location may be the interfacial region around the polar head groups of the surfactant (i.e. the sulfate groups for SDS or the phosphate groups for the DMPC and RLM systems). These results are also consistent with recent findings that water penetration into micellar structures[24] makes these regions very polar.

The nitronyl chemical shifts of the various nitrones may represent weighted, time-averaged values ($\delta_{mem/aq}$) due to chemical exchange between the membrane (e.g. SDS) and aqueous phases:[25]

$$\delta_{mem/aq} = (\chi_{mem}\delta_{mem} + \chi_{aq}\delta_{aq})/(\chi_{mem} + \chi_{aq}) \quad (2)$$

where $\delta_{mem/aq}$ = the observed, weighted, time-averaged nitronyl chemical shift, δ_{mem} and δ_{aq} = the nitronyl chemical shift in the purely membrane (e.g. SDS) and aqueous phases, respectively, and χ_{mem} and χ_{aq} = the mole fractions of nitrone in the purely membrane (e.g. SDS) and aqueous phases, respectively. One might therefore suspect that the high polarity reported by the various nitrones in SDS micelles could be accounted for by simply invoking a small nitrone concentration in a relatively nonpolar region of the 0.25 M SDS micelles with a large nitrone concentration in the 55 M bulk aqueous phase. This, however, does not appear to be the case because even the extremely hydrophobic nitrone, 4-DOPBN (water solubility $\sim 10^{-6}$ M) reports relatively high polarity for the microenvironment of its nitrone group in SDS micelles (Figure 3) despite the fact that this nitrone should not partition significantly out of the membrane phase into the bulk aqueous phase (χ_{aq} terms $\rightarrow$ 0, 2). The hydrocarbon chain of 4-DOPBN

(21) The EPR nitrogen hyperfine splittings of di-*tert*-butylaminoxyl (Knauer's a^N values) (in Gauss) are the following (cf. ref 7): tetrachloromethane (15.331), carbon disulfide (15.289), 1,4-dioxane (15.452), tetrahydrofuran (15.331), trichloromethane (15.863), dichloromethane (15.752), 2-propanone (15.527), dimethyl sulfoxide (15.962), nitromethane (15.759), 2-propanol (15.973), ethanol (16.030), methanol (16.210), water (17.175), 5 M LiBr (17.33) cf. footnote, Table V). Correlation with the nitronyl-^{13}C chemical shift of PBN gave an r^2 value of 0.91.

(22) The carbonyl-^{13}C chemical shifts (in ppm) for benzaldehyde were determined to be the following: tetramethylsilane (189.27), tetrahydrofuran (190.31), trichloromethane (192.45), 2-propanol (192.84), ethanol (193.43), methanol (194.24), water (199.17). Correlation with the nitronyl-^{13}C chemical shift of PBN yielded an r^2 value of 0.95.

(23) The organization of the membranes in liver microsomal preparation has been revealed by EPR and ^{31}P NMR to be mainly bilayer structures with some free phospholipid, cf.: Utsumi, H.; Murayama, J.-J.; Hamada, A. *Biochem. Pharmacol.* **1985**, *34*, 57–63 and references cited therein.

(24) Menger, F. M.; Boyer, B. J. *J. Am. Chem. Soc.* **1980**, *102*, 5936–5938.

(25) Chemical site exchange of the nitrones between the various membrane structures and the bulk aqueous phase is too fast to be detected by ^{1}H or ^{13}C NMR under these conditions. Values are available, however, by EPR where the time frame is inherently faster, for spin labels (ref 9f) and spin adducts (ref 4) of the same approximate size and polarity to the nitrones in question. These exchange rate constants generally fall into range of $\sim 10^6$ to 10^7 s^{-1}.

Table V. Nitronyl ^1H and ^{13}C NMR Chemical Shifts for PBN, 4-DOPBN, DMPO, and SCMPO in Various Solutions and Membrane Systems

solvent or membrane system	$E_{T(30)}$ value[8a]	PBN		4-DOPBN	DMPO		SCMPO
		^1H	^{13}C	^{13}C	^1H	^{13}C	^{13}C
tetramethylsilane	30.7	7.36	126.75		6.33	125.00	
cyclohexane	33.2	7.35	127.00			125.89	
tetrachloromethane	33.6	7.39	127.05	126.43	6.51	127.39	
carbon disulfide	33.9	7.34	127.28			131.57	
1,4-dioxane	36.0	7.55	128.10			133.53	
tetrahydrofuran	37.4	7.64	128.35	129.28		127.78	
trichloromethane	39.1	7.56	129.69	130.24	6.80	132.35	
dichloromethane	41.1	7.52	128.36	129.36		131.30	
2-propanone	42.2	7.76	128.99	130.43		129.28	
dimethyl sulfoxide	45.0	7.84	128.52			129.82	
nitromethane	46.3	7.70	130.67			137.53	
2-propanol	46.8	7.78	132.35	132.20		132.53	
ethanol	51.9	7.86	133.54	133.83		136.20	140.25
methanol	55.5	7.90	135.00	135.38		139.52	141.35
water	63.1	8.00	139.91	(137.80 est)	7.19	145.20	147.34
150 mM phosphate buffer (PB)	70		140.10				
5 M LiBr	(72)[a]	8.08	140.96		7.29	147.42	149.34
250 mM SDS		7.82	137.31	134.43		146.50	147.35
100 mM SDS			137.69				
50 mM SDS			138.17				
35 mM SDS			138.33				
25 mM SDS			138.63				
15 mM SDS			139.20				
10 mM SDS			139.97				
250 mM DMPC[b]			136.37	133.60			
100 mM DMPC			138.04				
50 mM DMPC			138.88				
25 mM DMPC			139.45				
10 mM DMPC			139.58				
RLM			139.49				

[a] Using the linear plot of the EPR nitrogen hyperfine splitting vs the solvent polarity parameter[7,8] and our value of 17.33 G for di-*tert*-butylaminoxyl, we estimate the $E_{T(30)}$ value for 5 M LiBr to be ~72 kcal/mol. [b] Because the phase transition temperature for DMPC is around 22 °C[15c] these spectra were recorded at 30 °C. The nitronyl ^{13}C chemical shift for PBN in 250 mM DMPC at 22 °C is 138.01 ppm.

probably orients itself toward the hydrocarbon core of the micelle with the nitrone group situated somewhere in the micellar periphery (Chart I). Therefore, chemical exchange of a given nitrone is expected to occur between a polar region of the micelle and the bulk aqueous phase with the extent of exchange governed by the hydrophobicity of the nitrone.

The nitronyl NMR chemical shift of PBN was also investigated in an aqueous solution that was high in salt content (i.e. 5 M LiBr) to simulate the most polar region of the headgroup zone of micelles and vesicles. These salt solutions cause an apparent increase in solvent polarity as judged by the solvent polarity probe, di-*tert*-butylaminoxyl[7,8,21] and $E_{T(30)}$. Both the ^1H and ^{13}C NMR nitronyl shifts of PBN in this medium exhibited values that were higher than those in pure water. In contrast, the effect of weaker salt solutions (e.g. 0.05 M phosphate buffer, the control solution for the DMPC vesicles) on the nitronyl shift in almost negligible.

The nitronyl ^{13}C chemical shift was found to be sensitive to the surfactant concentration. For instance, as the SDS concentration is gradually increased beyond the critical micelle concentration (CMC = 8.2 mM)[9f] the nitronyl ^{13}C NMR chemical shift for PBN decreases significantly relative to the value for pure water (Figure 4). This is indicative that micellization has commenced and that the nitrone is somehow solubilized within the micelle.

The time-averaged ^{13}C NMR chemical shifts for PBN in 10 mM DMPC vesicles is quite close to the value for the purely aqueous system or phosphate buffer. When the phospholipid concentration is systematically raised to 250 mM the nitronyl ^{13}C NMR chemical shift for PBN (Figure 1) is lowered significantly (136.37 ppm for 250 mM

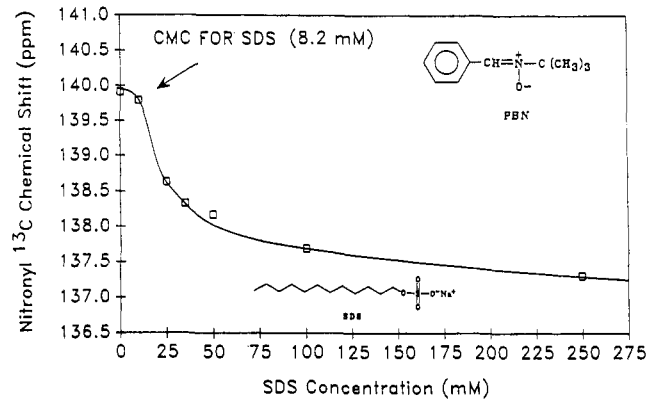


Figure 4. Sensitivity of the nitronyl ^{13}C NMR chemical shifts of PBN to the concentration of surfactant (SDS).

DMPC), indicating that sequestering of the nitrone into the vesicular membrane has occurred. By analogy to the SDS micelle results, PBN should prefer to be sequestered in the vesicular membrane phase, again in a polar region near the headgroup zone.

The nitronyl chemical shift for PBN in the RLM system (139.49 ppm) is significantly lower than that for the buffer control solution (140.10), which indicates that PBN has become solubilized in the RLM vesicular phase to some extent. It should be borne in mind that the nature of this vesicular phase may be quite different than the DMPC system due to the presence of considerable protein (40 mg/mL).

The ^{13}C plot for the aliphatic cyclic nitrone (DMPO) (Figure 5) exhibited more scatter (r^2 = 0.78) than the analogous PBN plot. The same general trend, however,

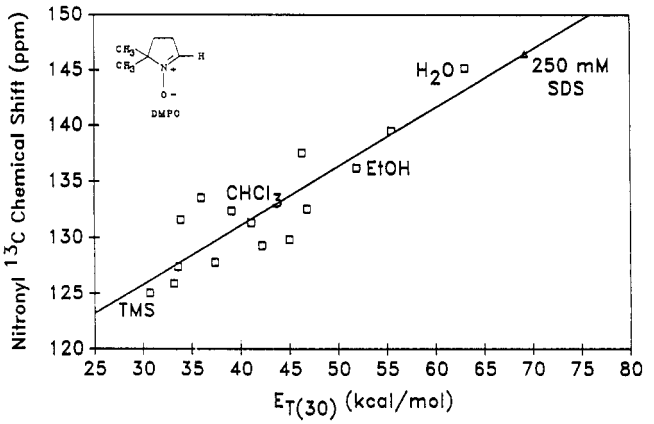


Figure 5. Plot of the nitronyl ^{13}C NMR chemical shifts of DMPO vs the solvent polarity parameter ($E_{T(30)}$).[8a] The $E_{T(30)}$ value for SDS was estimated from the regression line and is indicated by a triangle.

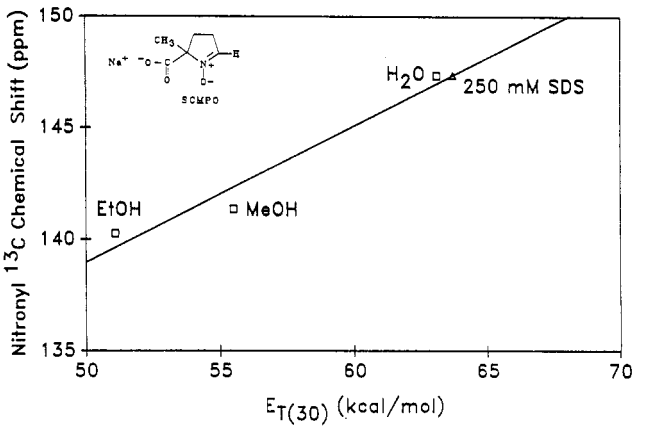


Figure 6. Plot of nitronyl ^{13}C NMR chemical shifts of SCMPO vs the solvent polarity parameter ($E_{T(30)}$).[8a] The $E_{T(30)}$ value for SDS was estimated from the regression line and is indicated by a triangle.

was observed: namely, an increase in solvent polarity induces an increase in the nitronyl chemical shift for both ^{1}H and ^{13}C (see Table III). Namely, that an increase in solvent polarity induces an increase in the nitronyl chemical shift for both ^{1}H and ^{13}C (see Table III). The chemical shift range (tetramethylsilane to water) for the ^{13}C plot is likewise much larger (~20 ppm) than the corresponding ^{1}H plot (0.8 ppm).

DMPO is considerably less hydrophobic than PBN (the octanol/water partition coefficients are 3.1 vs 15.1, respectively). Thus DMPO should partition significantly into the bulk aqueous phase in membrane-containing systems. This notion is borne out by the ^{13}C NMR results. In contrast to the nitronyl chemical shifts of the relatively hydrophobic nitrone (PBN and 4-DOPBN) which become smaller in SDS micelles relative to water (Figures 1 and 3), the nitronyl ^{13}C NMR chemical shift of DMPO actually becomes slightly larger (146.5 vs 145.2 ppm) (Figure 5). This finding suggests that the nitrone group of DMPO has a significant time-average residency in a region that is slightly more polar than water. A 5 M LiBr solution was found to cause a significant increase in the nitronyl ^{13}C chemical shift of DMPO (147.42 vs 145.20 ppm). Thus it appears that DMPO may locate itself in SDS micellar water pools in a region which resembles an aqueous solution with high salt content, i.e. near the sulfate head groups.

The plot of the nitronyl ^{13}C NMR chemical shift of SCMPO vs the solvent polarity parameter $E_{T(30)}$[8] is again reasonably linear (Figure 6 and Table IV). The paucity of data is due to the insolubility of SCMPO in all but the most polar solvents. The octanol/water partition coefficient for SCMPO is estimated to be $\sim 10^{-2}$. SCMPO is expected to remain in the bulk aqueous phase, and this is apparently observed. Steric repulsion between the carboxylate group of SCMPO and the sulfate group of SDS is expected to prevent penetration of this nitrone into the aggregate.

The nitronyl ^{1}H and ^{13}C NMR chemical shifts for PBN, 4-DOPBN, DMPO, and SCMPO, in various solutions with pure solvents as well as in the heterogeneous SDS micellar, DMPC phospholipid vesicular, and rat liver microsomal systems are collected in Table V.

Conclusions

In conclusion "the nitronyl chemical shift method" described above indicates that nitrones in general seek out the most polar regions of heterogeneous media and thus appear to function as amphiphilic molecules themselves. This tendency is exhibited to an increasing extent in the following order:

4-DOPBN < PBN < DMPO < SCMPO

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Registry No. PBN, 3376-24-7; 4-DOPBN, 80311-20-2; DMPO, 3317-61-1; SCMPO, 120386-02-9.