*MAGNETIC RESONANCE OF NITROXIDE PROBES IN MICELLE-CONTAINING SOLUTIONS**

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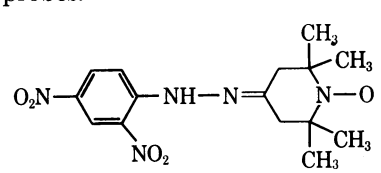
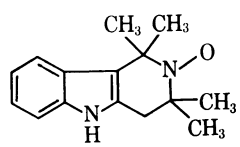
It is well known that many surface-active molecules aggregate into micelles at concentrations exceeding a certain value, the critical micelle concentration (cmc).[1] Studies of micelles are of interest because these relatively simple aggregates are model systems for biological membranes.[2] Useful new information about the solubilizing properties of micelles can be obtained using molecular probes capable of associating with the micelles. One such approach would be to introduce into solution a stable free radical probe which can be observed by electron spin resonance (ESR). Encouragement for using a free radical probe was provided by several earlier optical experiments which also used probes (in each case a dye).[1, 2] Nevertheless, our early attempts to investigate micelles using stable free radical probes were not successful because of the complex ESR spectra of the probes then available.[3] Recently Hoffmann and Henderson,[4] and Rozantzev and co-workers[5, 6] have synthesized a remarkable new class of nitroxide free radicals and we wish to report here the first investigation of micelles[7] using two of these stable free radicals, I and II, as probes.



I



II

Experimental.—The nitroxide free radical I is the 2,4-dinitrophenyl hydrazone of 2,2,6,6-tetramethyl-4-piperidone nitrogen oxide, and it was prepared as described previously.[5, 8] The free radical II, 2,2,4,4-tetramethyl-1,2,3,4-tetrahydro-γ-carboline-3-oxyl, was prepared according to the procedure of Rozantzev and Shapiro.[9] The sodium dodecyl sulfate (99+% NaDS) was obtained from Mann Research Laboratories and was used without further purification. The question of adequate purity of a surface-active molecule is always a difficult one. However, nearly all of the measurements were repeated using the average commercial 95 per cent NaDS and there was no appreciable difference in the data. Apparently the magnetic resonance measurements are not extremely sensive to impurities in the NaDS and further purification of the 99+ per cent NaDS for this work was not warranted.

All ESR spectra were recorded on a Varian V4502 X-band spectrometer. A Varian V-4532 dual cavity was used to determine the solubilities of I and II. In obtaining the rotational correlation time data, the probe concentration was 2×10^{-5} *M* when the NaDS concentration was above approximately 0.2 per cent. Below this point the concentration of probe was reduced because of solubility limitations and each solution was saturated with probe before use. The optical and nuclear magnetic resonance (NMR) data were obtained using a Cary model 15 spectrophotometer and a Varian A60 spectrometer, respectively.

Results.—Solubility and NMR data: Sodium dodecyl sulfate (NaDS) was chosen as the surface-active molecule because of the numerous studies that have been made on NaDS micelles. There is a considerable range in the values reported for the cmc of NaDS in water at 25°C and for the number of molecules per micelle at this concentration, but typical values are 0.23 per cent by weight NaDS (8.1×10^{-3} M) and 62 NaDS molecules/micelle, respectively.[10] If the solubilities of the probes are determined both in water and in aqueous NaDS concentrations well above the cmc, a measure of the affinity of the probes for the micelles can be obtained. These solubility data are given in Table 1. From Table 1, it can be seen that the solubility of I or II increases more than a hundredfold as the concentration of NaDS is raised from 0 to 5 per cent. It is clear from this increase in solubility that the probes strongly interact with the micelles. This was, in fact, why these two nitroxides were chosen as probes. Many other nitroxides are very soluble in water and the interpretation of the magnetic resonance spectra is complicated by the presence of significant concentrations of free radicals in both the aqueous and micelle phases.

The value of 5 per cent NaDS was chosen for a practical reason. This is the lowest concentration for which the NMR spectrum of NaDS is well resolved. The NMR spectrum of 5 per cent NaDS before and after the addition of 10^{-3} M nitroxide I is given in Figure 1. As would be expected at these low free radical concentrations, the H_2O line width is not measurably broadened. However, the widths of all three NaDS NMR lines are markedly increased. The same effect is observed after addition of nitroxide II. The logical question to ask is whether the magnitude of the observed broadening is what one would expect if all probes associate with the micelles. A precise answer to this question would require detailed knowledge of the nature of the relaxation processes as well as the structure of the micelles. We will therefore confine our approach to an approximate method based on the well-known studies of paramagnetic metal ions.

The contribution by a paramagnetic molecule to the proton line width, $\Delta\gamma'$, is

$$\Delta\gamma' = \frac{K\mu^2 N\eta}{kT}, \tag{1}$$

where μ^2 is the effective mean-square magnetic moment of the unpaired electron, N is the concentration of the paramagnetic centers, η is the local viscosity experienced by the paramagnetic center, and K is a constant.[11] Suppose that the translational motion of the paramagnetic molecules is random and is sufficiently rapid that all NaDS molecules experience the same average field. Assuming this to be the case, the broadening of the NaDS protons can be estimated using equation (1) and the line width data obtained from pure organic solutions containing the same para-

TABLE 1

SOLUBILITY OF NITROXIDE PROBES AT 23°C

Nitroxide	H_2O (M)	Dodecane (M)	5% NaDS (M)
I	5.0×10^{-6}	2.4×10^{-4}	1.1×10^{-3}
II	7.0×10^{-6}	1.2×10^{-4}	1.0×10^{-3}

The solubilities of I and II in water and dodecane are accurate to within $\pm 0.5 \times 10^{-6}$ M and $\pm 0.5 \times 10^{-4}$ M, respectively. The values in 5% NaDS are only representative since the amount dissolved depends to some extent on the time the solutions are in contact with the solid nitroxide. The values quoted above are for solutions stirred 2 hr with the powdered nitroxide. Up to 1.4×10^{-3} M has been obtained after stirring for 24 hr.

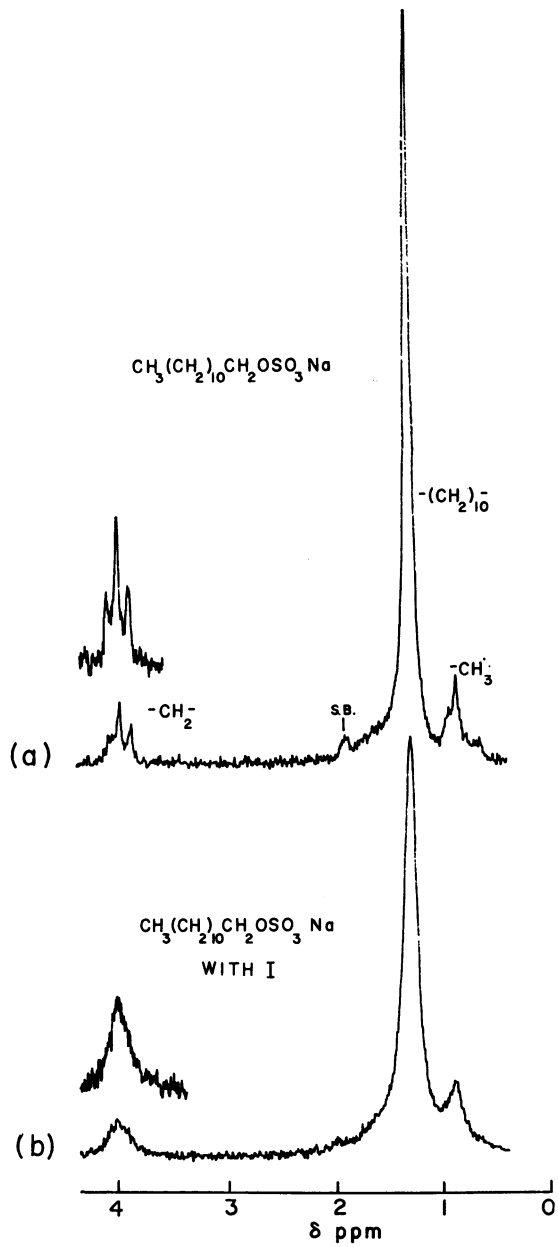


FIG. 1.—NMR spectrum at 23°C of a 5% NaDS-D_2O solution (*a*) before and (*b*) after addition of 1.1×10^{-3} *M* I. The TMS external standard signal occurs at 0 ppm.

magnetic molecule. The solvent chosen should closely resemble the long-chain NaDS molecule and must also be able to dissolve an equivalent concentration of the probe. The quantities K, μ^2, N, and T of equation (1) will then be approximately the same for the two solutions; the microscopic viscosity is the only variable remaining. From an extension of the theory of Debye, the local viscosity is directly proportional to the rotational correlation time, τ_c, of the probes.[11] A general discussion of τ_c appears in the following section. Here, however, we need only note

that τ_c is readily measured by ESR and thus sufficient information is available to allow the use of equation (1).

At 5 per cent NaDS almost all of the NaDS molecules are aggregated to form micelles. If the total concentration of I in this solution is adjusted to 0.53×10^{-3} M, the ratio of NaDS molecules to I is 330. If pure decanol is used for the line width study and the same ratio of I to long-chain molecules is maintained, then the probe concentration in decanol must be adjusted to 1.6×10^{-2} M. This is the maximum concentration of I that can be achieved without oversaturating the 23°C decanol solution. Turning now to the NMR data, only the intense $-(CH_2)_8-$ line on which accurate measurements can be made will be considered. The full width at half height of this line in pure decanol and in a decanol solution containing 1.6×10^{-2} M I is 5.9 and 9.2 cps, respectively. The width of the corresponding peak in the NaDS solution without I is 4.3 cps. The values of τ_c at 23°C for I in decanol and 5 per cent NaDS were found by ESR to be 4.2×10^{-10} sec and 6.0×10^{-10} sec, respectively. Therefore, the width of the NMR line of NaDS in the aqueous solution containing 0.53×10^{-3} M I is predicted to be $4.3 + (9.2 - 5.9)\ (6.0/4.2) = 9.0$ cps. This value is in good agreement with the observed value of 8.0 cps ± 0.5 cps. Other concentrations of I in 5 per cent NaDS ranging from 0.27×10^{-3} M to 0.53×10^{-3} M also yielded values for the broadening, $\Delta\gamma'$, of the NaDS line which are consistantly 70–90 per cent of the value predicted. These results are gratifying because if the nitroxide radical did not associate with the micelles, there would be no detectable broadening. However, care should be exercised when extending the predictions above 0.53×10^{-3} M I. The NaDS broadening does not continue to increase as rapidly with the nitroxide concentration as the decanol broadening predicts. In fact, at 1.07×10^{-3} M I the NaDS broadening is about 40 per cent of the value predicted. A detailed study of the proton relaxation processes is required before definite conclusions can be reached from the NMR data regarding the dynamic processes. It can be said at this point, however, that the amount of broadening is on the order of what is predicted assuming the probes associate with the micelles. Thus, the NMR results for I are consistent with the solubility data for I. Similar behavior was exhibited by II.

ESR spectra and rotational motion of I and II: The ESR spectrum of I in water or dodecane consists of three lines of nearly equal width (Fig. 2*a*). This hyperfine pattern is characteristic of a variety of rapidly tumbling nitroxides. As shown in Figure 2*b*, however, the spectrum of I in water containing 5 per cent NaDS is markedly broadened. The same effect is observed for radical II. This unequal broadening of the three hyperfine lines is caused by a decrease in the rate of tumbling of the probe. The rate of tumbling is quantitatively described by a rotational correlation time, τ_c. Several authors have developed theories relating τ_c to the spectral line widths.[12, 13] Fortunately, the tumbling rates encountered in this work fall within an optimal range. The appropriate equations are[7, 13]

$$\tau_c^{(1)} = \left[\frac{W_1}{W_0} - \frac{W_{-1}}{W_0}\right]\left[-\frac{8b\Delta\gamma H}{15\pi\sqrt{3}\,W_0}\right]^{-1}, \tag{2}$$

$$\tau_c^{(2)} = \left[\frac{W_1}{W_0} + \frac{W_{-1}}{W_0} - 2\right]\left[\frac{b^2}{4\pi\sqrt{3}\,W_0}\right]^{-1}, \tag{3}$$

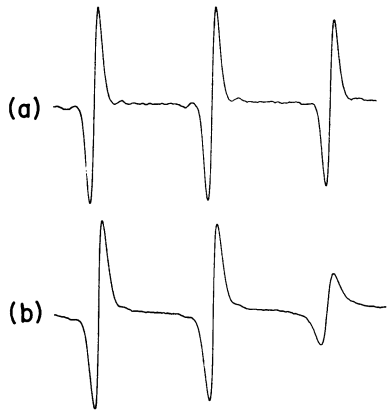


FIG. 2.—X-band ESR spectrum of nitroxide I at 23°C in (*a*) water and (*b*) water containing 5% NaDS. The corresponding spectra of radical II are similar to (*a*) and (*b*) and are not shown. The distance between the outermost lines of (*a*) is 32.3 gauss.

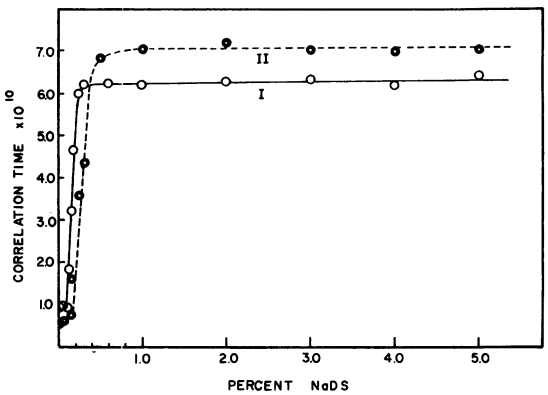


FIG. 3.—Plot of the rotational correlation time of nitroxides I and II in 23°C aqueous solution vs. NaDS concentration.

where $b = 4\pi(A - B)/3$, $\Delta\gamma = |\beta|\ [g_{zz} - (g_{xx} + g_{yy})/2]\hbar^{-1}$, and W_1, W_0, and W_{-1} are the peak-to-peak widths (Mc/sec) of the low, middle, and high field first-derivative lines, respectively. The quantity H is the laboratory magnetic field and A, B, g_{xx}, g_{yy}, g_{zz} are the parameters of the spin Hamiltonian. It is worth noting that equation (2) and equation (3) each yield a value of τ_c, and these two values, according to the theory, should be equal. The ratios W_1/W_0 and W_{-1}/W_0 may either be evaluated directly from the line widths or may be replaced by the ratios $(h_0/h_1)^{1/2}$ and $(h_0/h_{-1})^{1/2}$ where h_1, h_0, h_{-1} are the corresponding heights of the first-derivative lines. The latter procedure was used by Stone, Buckman, Nordio, and McConnell in their study of poly-L-lysine[7] and it is the procedure used here. The following three assumptions are also made: (*a*) the Hamiltonian parameters for I and II are the same as those reported by Griffith, Cornell, and McConnell[14] for the very similar di-tert-butyl nitroxide radical (hence $b = 3.06 \times 10^8$ sec^{-1} and $\Delta\gamma = 4.22 \times 10^4$ sec^{-1} gauss^{-1}); (*b*) the line shape is precisely Lorentzian; and (*c*) the motion is isotropic. The first two approximations do not significantly affect our conclusions. The third assumption, however, deserves further comment and is discussed later in conjunction with the splitting and optical data.

The resulting plot of the rotational correlation time as a function of the NaDS concentration is given in Figure 3. The two values of τ_c at any given concentration of NaDS differed at most by only 10 per cent, and therefore only the average of the two values is plotted in Figure 3. It is immediately apparent from Figure 3 that a sharp change in the rotational correlation time occurs in the region of the cmc. At points well below the cmc the nitroxide probes tumble at a rate characteristic of rotation in pure water. At points significantly above the cmc, the tumbling rate is slower but it is again nearly independent of the NaDS concentration over the range of concentrations investigated. The most logical explanation of these data is that the probes, initially tumbling rapidly in water, are slowed down above the cmc because of interactions with the micelles.

Several models of solubilization have been suggested.[1, 2] In one case the probe (or foreign body) is visualized as being adsorbed on the surface of the micelle; in another model the probes are oriented radially in the micelle with their polar groups fixed near the surface. In still a third model the probes are considered to be dissolved in the hydrocarbon interior of the micelle away from the polar groups. One can, in principle, distinguish between these models by comparing the experimental rotational correlation times with values from the simple Stokes law relation[11, 15]

$$\tau_c = \frac{4\pi\eta a^3}{3kT} \tag{4}$$

using appropriate values of the particle radius, a. Consider radical I, for example. Assuming a nitroxide density of 0.9 (a = 5.4 Å) and using the macroscopic viscosities of the solvents, the Stokes law values of τ_c for I in water and dodecane are 1.6×10^{-10} sec and 1.8×10^{-10} sec, respectively. By contrast, the Stokes law value of τ_c for I bound to a rigid spherical NaDS micelle of radius 22 Å is 1.1×10^{-8} sec. The experimental values of τ_c in water, dodecane, and for aqueous solutions of NaDS above the cmc are 0.9×10^{-10} sec, 0.7×10^{-10} sec, and 6.0×10^{-10} sec, respectively. The first two values are in good agreement with the corresponding Stokes law values but the third number is 18 times smaller than the corresponding Stokes law value of τ_c. From this we conclude that the probe is not tumbling at the rate predicted for a rigid micelle and therefore the probe cannot be considered as being adsorbed on the surface of a hypothetical spherical (or ellipsoidal, etc.) micelle. The same conclusion is reached for II since the tumbling rates of I and II are very similar. Thus the rotational correlation time data have eliminated the first simple model of solubilization suggested above. The second and third models cannot be tested adequately using the rotational correlation time data since both models allow relatively rapid motion of the probes. These two models are discussed below in connection with the splitting and optical data.

Solvent effects on ESR coupling constants and optical spectra: It is easily seen from the structures of I and II that each probe consists of two distinct sites, one paramagnetic site and one aromatic chromophore. The paramagnetic site is, of course, the nitroxide (N-O) and the aromatic site is either a 2,4-dinitrophenyl moiety (I) or an indole group (II). In principle, the polarity of the environment of each site can be determined by independent methods. Il'yasov[16] has reported a solvent effect on the ESR splittings of nitroxides, and the solvent shifts of optical spectra of aromatic chromophores are well known. An interesting question to ask, therefore, is whether the local environments of both ends of the probe are the same. The ESR line width data suggest that this is the case since the rapid motion would tend to average the environments of the two sites. One could argue, however, that the rapid tumbling is not completely isotropic and that the 2,4-dinitrophenyl group of I is on the average closer to the polar head groups of the NaDS molecules. Similar arguments could be made for II and this question is of obvious importance.

The nitroxide coupling constants measured with the probes dissolved in water, dodecane, and aqueous NaDS solutions are given in Table 2. In Table 3, the solvent dependence is given for the maximum in the optical absorption spectrum of

TABLE 2

NITROXIDE HYPERFINE COUPLING CONSTANTS AT 23°C

Nitroxide	In H_2O (gauss)	In dodecane (gauss)	In 5% NaDS (gauss)
I	16.16	14.30	15.72
II	16.97	15.15	16.60

All coupling constants were measured between the low field and middle field peaks of the ESR spectrum. A relative error of ±0.05 gauss between the measurements is assigned. Absolute values are based on the 13.0-gauss coupling constant of peroxylamine disulphonate.

TABLE 3

OPTICAL ABSORPTION MAXIMA AT 23°C FOR NITROXIDE I

In H_2O (Å)	In dodecane (Å)	In 5% NaDS (Å)
3690	3440	3640

These values are believed to be accurate to within ±10 Å.

I. Although the data are quoted at 5 per cent NaDS, the ESR and optical data were essentially independent of the NaDS concentration over the range investigated, i.e., 2 to 5 per cent NaDS. The coupling constants of the solubilized nitroxides lie between the extreme values observed for the probes in water and dodecane, but they are much closer to the values observed in water. Crudely speaking, the polarity of the local environment of the paramagnetic site of I in the 5 per cent NaDS solution is 78 per cent that of water and only 22 per cent that of dodecane. From Table 3, the corresponding numbers at the aromatic site of I are 80 per cent water and 20 per cent dodecane and it is evident that the two sites of I experience the same average local environment. We conclude that radical I is not oriented in the micelle, and that the second simple model for solubilization proposed above is not applicable without modification. Furthermore, the third model, in which the probe is dissolved in a purely hydrocarbon environment, is clearly not correct. The three simple models that are most frequently proposed are, for this system at least, very poor approximations. These data suggest that a new model be considered for the solubilization of I and II. Such a model can be formulated as a dynamic association with an aggregate of NaDS molecules in which the probe preserves a random spatial orientation and experiences a relatively polar time-averaged environment. The tumbling rate of the probe is rapid, only six times slower than in pure water.

It is well to recall from the solubility data of Table 1 that the ratio of probes associated with the micelles to the probes in the aqueous phase is on the order of 100:1 for the 5 per cent NaDS solution. Therefore, the effect of the probes which are in a purely aqueous environment does not significantly complicate the interpretation of the splitting and optical data. Another possible source of difficulty, partial decomposition of the probe, has been ruled out by carefully observing the spectra over long periods of time and by studying the effects of the most likely impurity, 2,2,6,6-tetramethyl-4-piperidone nitrogen oxide. Similar arguments hold for radical II. The ESR splitting data of II are very similar to those of radical I. In the case of radical II, however, the solvent dependence of the optical spectrum is very small and no information regarding the environment of the aromatic site is obtained.

Perhaps the most interesting aspect of this study is the emphasis on the dynamic nature of solubilization. The ESR results, and to some extent the NMR data,

serve to illustrate that the static models for the incorporation of foreign molecules in micelles have limited significance. The combination of ESR and NMR techniques to observe both the probe and the environment of the probe should be of use in other solubilization studies and perhaps in transport problems where the rotational freedom and relative positions of the molecules are also of significance.

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