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Proton Magnetic Resonance Relaxation Studies on the Structure of Mixed Micelles of Triton X-100 and Dimyristoylphosphatidylcholine†

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ABSTRACT: Proton magnetic resonance and gel chromatographic studies on mixtures of phospholipid and the nonionic surfactant Triton X-100 have shown that at temperatures above the thermotropic phase transition of the phospholipid and below the cloud point of Triton, mixed micelles are present at molar ratios above about 2:1 Triton/phospholipid. Proton T_1 and T_2* (from line widths) relaxation times are reported for protons in Triton micelles and in mixed micelles of Triton and dimyristoylphosphatidylcholine at a molar ratio of 3:1 Triton/phospholipid. The T_1 values and their temperature dependence and the activation energies of the various Triton proton groups appear to reflect internal motions of the Triton molecules in the micelle. Measurements of the T_1/T_2* ratio and frequency dependence (55–220 MHz) suggest that the hydrophobic *tert*-butyl group in Triton is observed under extreme narrowing conditions. The T_1 and T_2* values of Triton are unchanged in the presence of phosphatidylcholine. The T_1 values of various protons of dimyristoylphosphatidylcholine in mixed micelles are similar to those reported for the phospholipid in sonicated vesicles, which are used as membrane models, and presumably the same coupled trans–gauche motions dominate. The T_2* values for the terminal methyl and choline methyl protons in the phospholipid are longer than those reported for these groups in vesicles. Hence, the motion of the phospholipid in the mixed micelles appears to be less restricted than in vesicles. T_1 measurements in H_2O/D_2O mixtures are consistent with the idea that water does not penetrate the hydrophobic core of the mixed micelles, while water does solvate the polar oxyethylene and choline methyl groups. Titration with Mn^{2+} confirms that the oxyethylene and choline methyl groups are on the exterior of the mixed micelle while the hydrophobic groups are located in the micellar interior.

Previous ^{1}H nuclear magnetic resonance (NMR) (Dennis and Owens, 1973; Ribeiro and Dennis, 1974a) and gel chromatographic studies (Dennis, 1974a) have led to the suggestion that phosphatidylcholine and the nonionic surfactant Triton X-100 form mixed micellar structures at high molar ratios of Triton/phospholipid. These mixed micelles provide one form of the phospholipid which lipolytic enzymes such as phospholipase A_2 can utilize as substrate (Dennis, 1973) and the phospholipase A_2–dipalmitoylphosphatidylcholine–Triton X-100 system provides an artificial, characterizable system for studying the effect of thermotropic phase transitions and lipid phase separations on biological activity (Dennis, 1974b). Mixed micelles of phospholipid and surfactant are also important in membrane studies since they are formed when Triton X-100 and other surfactants are employed in the solubilization of membrane-bound proteins (Helenius and Söderlund, 1973; Makino et al., 1973).

Using continuous wave ^{1}H NMR techniques, we have shown that the phospholipid molecules in mixed micelles give rise to full or nearly full intensities and narrow line widths (Dennis and Owens, 1973; Ribeiro and Dennis, 1974a), whereas unsonicated dispersions of phospholipid which have a multibilayer structure do not (Penkett et al., 1968; Chan et al., 1973). Furthermore, the Triton molecules in Triton micelles and mixed micelles have similar intensities and line widths (Dennis and Owens, 1973; Ribeiro and Dennis, 1974a). Preliminary T_1[1] measurements have suggested that the T_1 relaxation times are also similar in Triton micelles and mixed micelles (Ribeiro and Dennis,

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[1] Abbreviations used are: diacylphosphatidylcholine, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine; TSP, sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$; TMS, tetramethylsilane; cmc, critical micelle concentration; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time.

1975). Initial experiments were conducted on phosphatidylcholine isolated from egg yolk (Dennis and Owens, 1973), which has a heterogeneous population of fatty acid side chains, and on synthetic dipalmitoylphosphatidylcholine (Ribeiro and Dennis, 1974a), which is homogeneous with respect to fatty acid chains, but which forms mixed micelles poorly at lower temperatures because it has a thermotropic phase transition at 41° (Phillips et al., 1969). Furthermore, Triton X-100 exhibits a cloud point (phase separation) at about 64° (Maclay, 1956), and phospholipid lowers the cloud point significantly (Ribeiro and Dennis, 1974b) making it difficult to study mixed micelles at temperatures greater than about 45° (Ribeiro and Dennis, 1974a). Because knowledge of the temperature dependence of various ^{1}H NMR parameters is necessary in interpreting studies on the structure of mixed micelles, we have now conducted more detailed ^{1}H NMR studies on mixed micelles prepared from Triton X-100 and synthetic dimyristoylphosphatidylcholine. This phospholipid is homogeneous with respect to its fatty acid chains, but undergoes its thermotropic phase transition at about 23° (Phillips et al., 1969; Hinz and Sturtevant, 1972; Melchior and Morowitz, 1972). A preliminary report of some of these results has been presented (Dennis and Ribeiro, 1974).

Experimental Procedure

Dimyristoylphosphatidylcholine was obtained from Calbiochem (lots 300018, 300326, and 400203). Thin-layer chromatography of the phospholipid in chloroform–methanol–water (65:24:4 v/v) on silica gel G using 0.25 mm × 20 cm × 20 cm glass plates gave one spot. Optical rotation measurements on a Perkin-Elmer Model 141 polarimeter at 25° as 4% solutions in $CHCl_3$ yielded an $[\alpha]_D$ of +6.4, +5.5, and +7.0° for lots 300018, 300326, and 400203, respectively. Product literature specified a value of +5.8°. The optical purity of synthetic phosphatidylcholines is discussed elsewhere (Dennis, 1973). Triton X-100 (Rohm and Haas) is a polydisperse (Becher, 1967; Enyeart, 1967) preparation of *p-tert*-octylphenoxypolyethoxyethanols with an average chain length of about 9.5 oxyethylene units and may contain some heterogeneity in the hydrophobic portion (Enyeart, 1967). Concentrations are expressed in terms of an average monomer molecular weight of 624. The cmc of Triton X-100 is about 0.2–0.3 m*M* and depends somewhat on conditions (Crook et al., 1963; Ray and Némethy, 1971). All experiments reported here were conducted at sufficiently high concentrations of Triton that the monomer concentration is negligible and Triton can be assumed to be micellar. All other chemicals were of reagent grade. Samples were prepared by adding solutions of Triton X-100 in D_2O (or containing some H_2O where indicated) to dry phospholipid; mixing was achieved by a few strokes with a Potter–Elvejhem homogenizer. For measurement of relaxation times, the samples were deoxygenated with several freeze–pump–thaw cycles or by flushing with argon. Samples treated by both methods gave similar T_1 values.

Continuous wave ^{1}H NMR spectra were obtained on a Varian HR-220 spectrometer operating at 220 MHz as described previously (Ribeiro and Dennis, 1974a). Pulsed ^{1}H NMR studies were conducted on an extensively modified Varian HR-60 spectrometer operating at 55 MHz and described elsewhere (Vold et al., 1973), a JEOL PFT-100 Fourier transform system operating at 100 MHz and equipped with a Nicolet 1085 computer and disk, or on a Varian HR-220 spectrometer equipped with a Transform Technology TT-100 Fourier transform system. Experiments were conducted on the 100-MHz spectrometer, except where noted. Sample temperatures were determined from the chemical shift of an ethylene glycol sample, except at 55 MHz where a thermocouple was employed. Spin–lattice relaxation times (T_1) were determined by the partially relaxed Fourier transform (PRFT) method of Vold et al. (1968) using a 180°–τ–90° pulse sequence. The delay time was always at least 8 × T_1 for the longest T_1 of the sample (excluding HOD) and 24–29 τ values were employed in every relaxation experiment reported here. Peak heights (I_τ) were hand-measured on expanded spectra for every resonance line on all spectra obtained. Linear plots of log ($I_\infty - I_\tau$) vs. τ were obtained for all nonoverlapping peaks and the T_1 values were obtained from the slope. For nonoverlapping peaks, excellent fits to a least-squares program were obtained as the correlation coefficient was +0.99 for the bulk of the experiments (Figure 1A). The oxyethylene protons (peak e) give rise to several chemically shifted, but slightly overlapping lines (see Results); linear plots were obtained for several components, but because of resolution limits the fits were not as good and the correlation coefficient averaged about +0.97. For the terminal methyl protons of the phospholipid (peak x), which is a triplet, the intensity of the center line was used to obtain the T_1 values. The phospholipid methylene protons (peak y) and the Triton dimethyl protons (peak b) have approximately the same chemical shift (see Figure 2); individual T_1 values were obtainable by the method of "curve stripping" (Noggle and Schirmer, 1971) as shown in Figure 1B. Errors in the T_1 values reported here are estimated to vary between ±3 and 10%, except for very small peaks and data obtained at 220 MHz where the error was slightly greater. Line widths ($\Delta\nu_{1/2}$) were measured as the full width at half-height maximum intensity on expanded spectra; field inhomogeneity was taken to be the line width of the HOD peak and this was substracted from the reported results. T_2* values were obtained from the corrected line widths and the expression $\Delta\nu_{1/2} = 1/\pi T_2$*. Error in the T_2* values is estimated to be about ±10% for singlet peaks and ±20% for the center line of the peak x triplet.

Results

Relaxation Times of Mixed Micelles. Spin–lattice relaxation times were determined for various resonances in Triton micelles and mixed micelles with dimyristoylphosphatidylcholine at a molar ratio of 3:1 Triton/phospholipid over the temperature range 25–43°. The determination of some T_1 values is shown in Figure 1, and the resulting T_1 values are shown in Arrhenius plots in Figure 3. The T_1 values for the Triton protons are identical within experimental error for Triton micelles and mixed micelles (Figure 3A and B). The resulting plots are linear and the activation energies are given in Table I. Arrhenius plots of T_2* values calculated from the line widths of selected singlet peaks in Triton micelles and mixed micelles are shown in Figure 4 and the activation energies are included in Table I.

Line widths (and T_2* values calculated from them) are similar for the *tert*-butyl group at the hydrophobic end of Triton, peak a, in Triton micelles and mixed micelles. The T_1 value for this peak is not frequency dependent within experimental error, although other Triton protons may be and the hydrophobic phospholipid protons appear to be as shown in Table II. T_1/T_2* of peak a is small and similar in micelles and mixed micelles at both 100 and 220 MHz as

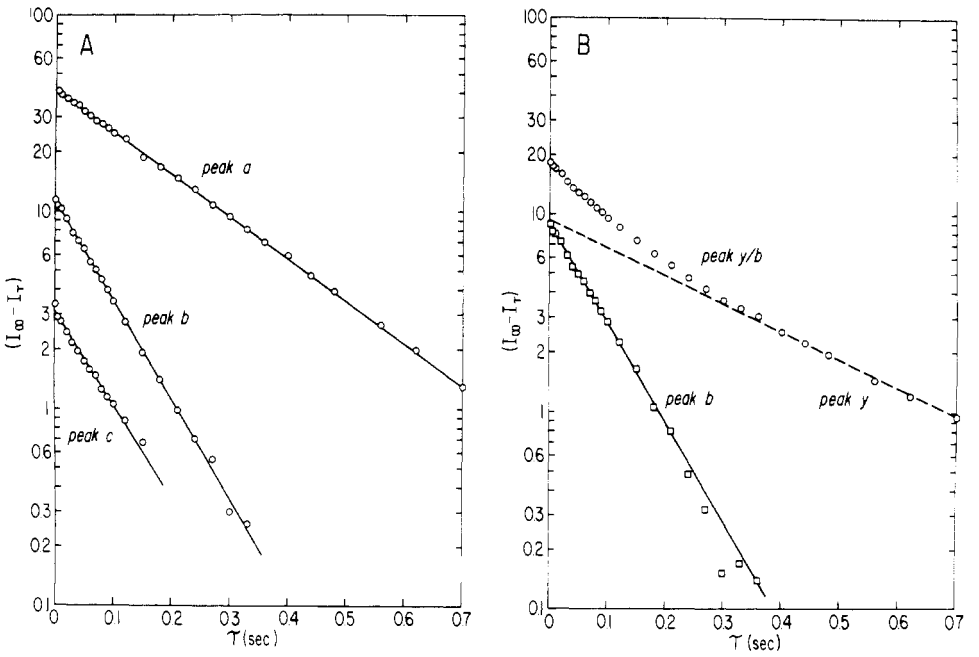

FIGURE 1: (A) Typical plots of log $(I_\infty - I_\tau)$ vs. τ for the alkyl resonances in micelles of 100 mM Triton X-100 in D_2O at 30°. See Figure 2 for identity of peaks. (B) Curve stripping of overlapping resonances, peak y of phospholipid and peak b of Triton, for mixed micelles of 100 mM Triton X-100 and 33 mM dimyristoylphosphatidylcholine in D_2O at 30°. The shorter T_1 value was assigned to peak b on the assumption that the T_1 value of this group is similar in the presence of phospholipid as is the case with peak a as will be shown in Figure 3.

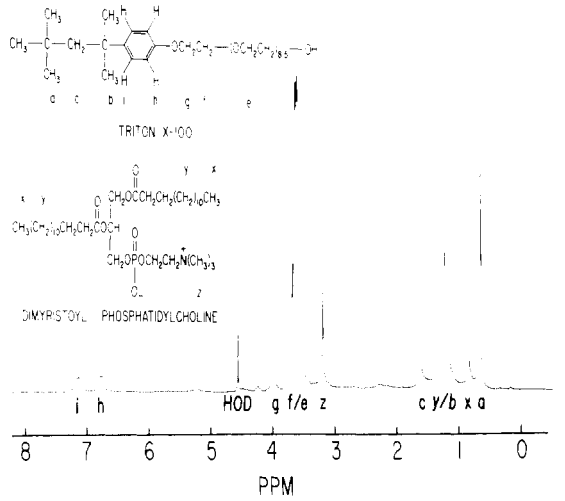

FIGURE 2: The 220-MHz Fourier transform ^1H NMR spectrum recorded at 40° of mixed micelles of 100 mM Triton X-100 and 33 mM dimyristoylphosphatidylcholine in D_2O relative to TSP. Peaks were assigned previously (Dennis and Owens, 1973). This spectrum was obtained from a 180°-τ-90° pulse sequence with τ = 20 sec; at this pulse spacing the HOD resonance was only partially recovered.

Table I: Activation Energies in Mixed Micelles.[a]

Peak	E_a (T_1)	E_a (T_2*)
a	4.1	4.1
b	5.4	5.3[b]
e-highest field	3.2	
e-lowest field	4.9	
h	2.6	
i	2.4	
x	4.7	
y	2.2[c]	
z	3.9	3.0

[a] E_a (kcal/mol) from data in Figures 3 and 4. For Triton peaks, the activation energies are taken from the average line determined both with and without phospholipid present. [b] Triton X-100 micelles alone. [c] This activation energy may have greater error than the others due to greater inaccuracies in obtaining T_1 for this group by the curve-stripping procedure. Also, it should be noted that the T_1 values for the methylene protons may actually reflect an average value for the various methylene groups in the fatty acid chains.

shown in Table III. T_1 values and line widths or T_2* values for phosphatidylcholine in mixed micelles are compared with reported values for unsonicated preparations of phospholipid (multibilayers) and sonicated preparations (vesicles) in Table III; this comparison will be considered further in the Discussion.

The T_1 values of selected resonance lines in Triton micelles and mixed micelles at several concentrations are shown in Table IV. The T_1 values are identical within experimental error over this concentration range. Reported values of the viscosity of Triton X-100 solutions in the concentration range of this study suggest that the T_1 values are not affected by changes in viscosity.

The effect of various amounts of Mn^{2+} on the line width of selected resonances in the mixed micelles is shown in Figure 5 and on the spin-lattice relaxation times is shown in Table V. The line widths and T_1 values of the HOD, choline methyl (peak z), and the oxyethylene resonance lines are greatly affected by the addition of Mn^{2+}. On the other hand, the line widths and T_1 values of the resonance lines that one would associate with hydrophobic groups are essentially unaffected until quite high concentrations of paramagnetic ions. This experiment also suggests that the relaxation times reported here without added Mn^{2+} are presumably not due to paramagnetic relaxation mechanisms.

Solvent Dependence of Relaxation Times. T_1 values for various resonance lines in Triton micelles and mixed micelles in the presence of various amounts of H_2O in the D_2O solvent are given in Table VI. With the addition of H_2O,

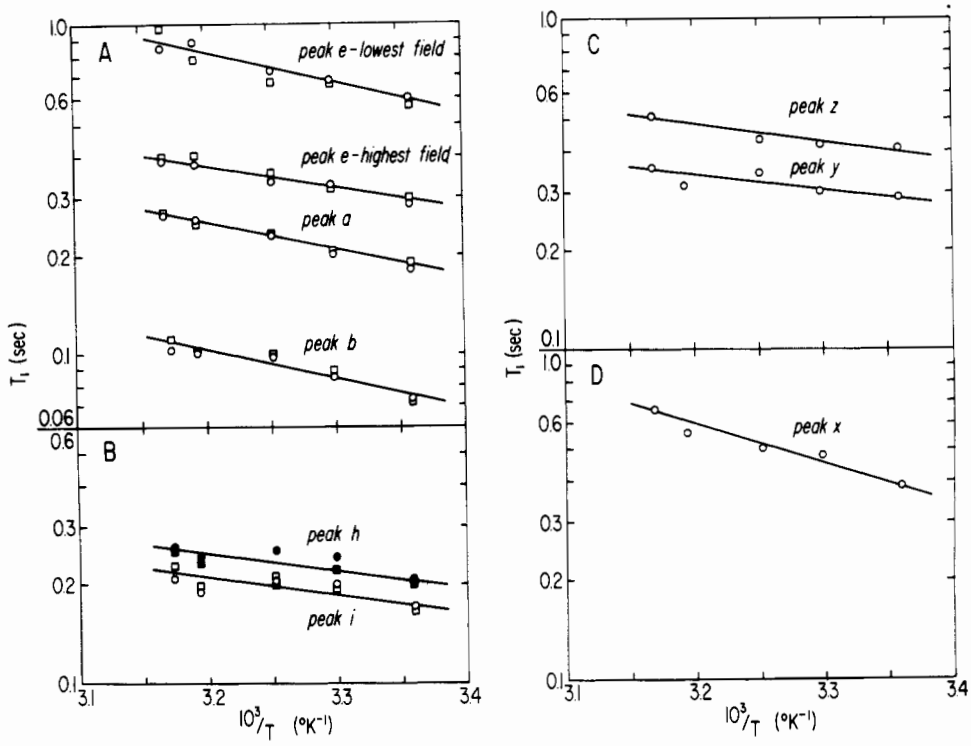

FIGURE 3: (A,B) Arrhenius plots of T_1 values for various resonances in 100 mM Triton X-100 (○) and for the same peaks in the presence of 33 mM dimyristoylphosphatidylcholine (□). Peaks h and i are doublets; the average T_1 values for the two components are shown. Peak e consists of several chemically shifted lines; the T_1 values appear to increase in going from the upfield to the downfield components. Only T_1 values for the component furthest upfield and furthest downfield are shown. (C,D) Arrhenius plots of the various phospholipid peaks for the mixed micelles of Triton and phospholipid.

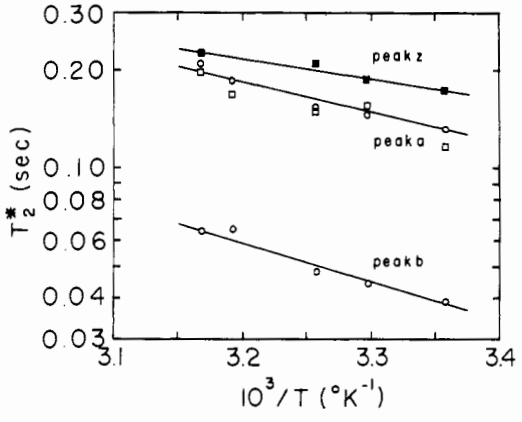

FIGURE 4: Arrhenius plots of T_2* values for selected resonances in 100 mM Triton X-100 (○) and in the presence of 33 mM dimyristoylphosphatidylcholine (□).

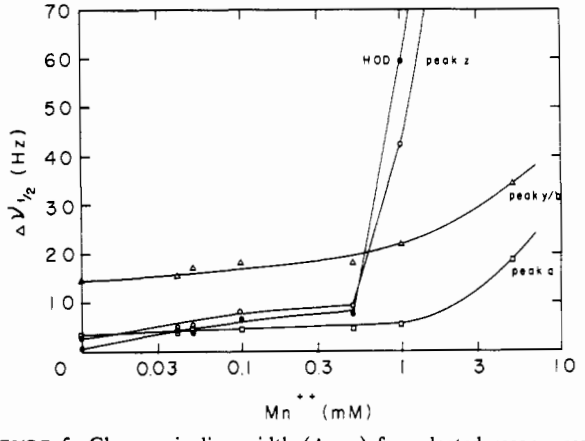

FIGURE 5: Changes in line width ($\Delta\nu_{1/2}$) for selected resonances of mixed micelles composed of 100 mM Triton X-100 and 33 mM dimyristoylphosphatidylcholine upon titration with $MnCl_2$ at 34°. Field inhomogeneity was not subtracted from the reported $\Delta\nu_{1/2}$. The observed line width of peak y/b is not a true width due to the overlap of the two peaks.

the T_1 values do not change significantly for the groups in the hydrophobic region, but they appear to decrease for the choline methyl group (peak z) in the hydrophilic region of the mixed micelles. Unfortunately, due to the overlap of lines in the oxyethylene band (peak e) (see below), the experimental error is too large to tell from our data whether the T_1 values of these protons change in the presence of H_2O.

The ^1H NMR spectra of Triton X-100 in D_2O and $CDCl_3$ are shown in Figure 6. In $CDCl_3$ and in CD_3OD (spectrum not shown), the spectra are much sharper than in D_2O, presumably reflecting Triton molecules in inverse micelles or in monomeric form rather than in micelles. In D_2O, where Triton X-100 forms micelles, the oxyethylene protons (peak e) give rise to several chemically shifted and partially overlapping resonance lines and they appear asymmetrically distributed as shown in Figure 7. We could detect up to 13 components in a previously published continuous wave spectrum at 220 MHz (Ribeiro and Dennis, 1974a), but can obtain reliable T_1 values for only 5–7 components at 100 MHz and 10–11 components at 220 MHz. There appears to be a trend for the T_1 values to increase in going from the high-field component to the lowest field component. This asymmetric band has also been observed at 220 MHz by Podo et al. (1973), and these workers have assigned the sharp spike at the low-field end to the two methylene protons at the hydroxyl end of the final oxyethy-

Table II: Frequency Dependence of T_1 Relaxation Times for Micelles of Triton X-100 and Mixed Micelles with Phosphatidylcholine.[a]

Peak	55 MHz Triton	100 MHz Triton	100 MHz Triton + PC	220 MHz Triton	220 MHz Triton + PC
a	0.23	0.26	0.26	0.27	0.26
b		0.10	0.11	0.12	0.11
c		0.10	0.13	0.12	0.15
e[b]					
1	0.43[c]	0.38	0.37	0.43	0.45
2		0.47	0.39	0.46	0.48
3		0.55		0.53	0.47
4		0.63	0.56	0.58	0.54
5		0.69		0.53	
6				0.64	0.62
7		0.89	0.79	0.57	0.62
8				0.79	0.75
9				0.66	0.96
10				0.82	0.82
11				0.97	1.2
f		0.38		0.37	
g		0.17		0.25	
h[d]		0.24	0.22	0.38	0.35
		0.25	0.24	0.39	0.40
i[d]		0.18	0.19	0.35	0.35
		0.19	0.20	0.33	0.33
x			0.56		0.68
y			0.31		0.43
z			0.49		0.51

[a] T_1 values (sec) at 40° for a sample of 100 m*M* Triton X-100 containing 33 m*M* dimyristoylphosphatidylcholine where indicated. [b] Resolvable resonance lines in peak e at 100 MHz are numbered 1–7 in the downfield direction and at 220 MHz are numbered 1–11. Peak 7 at 100 MHz and peak 11 at 220 MHz represent the same sharp spike at the low-field side of peak e. [c] Average value for peak e due to lack of resolution at 55 MHz. [d] The T_1 values for each component of the doublet for peaks h and i are listed with upfield component first.

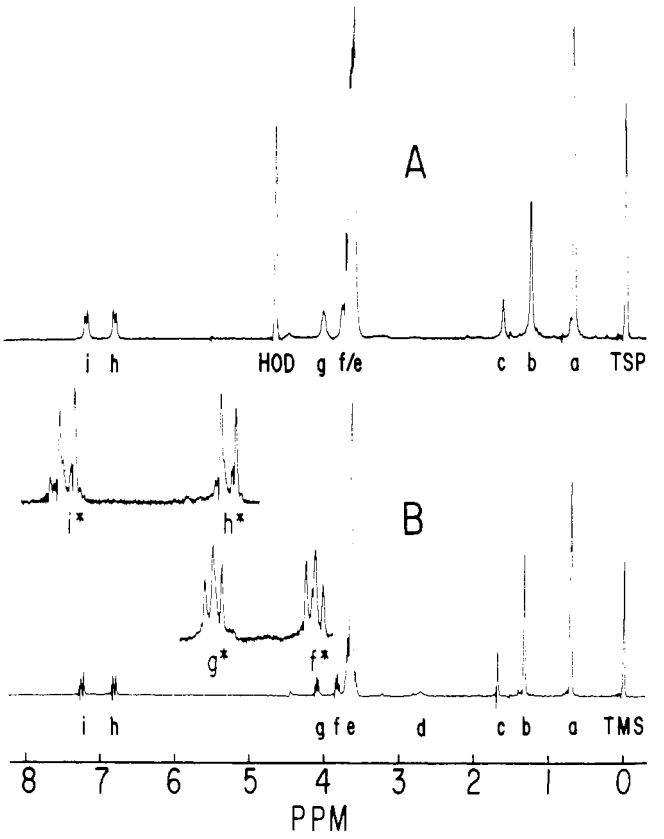

FIGURE 6: The 220-MHz continuous wave ^{1}H NMR spectra recorded at 2500 Hz sweep width and 37° of 100 m*M* Triton X-100 in (A) D_2O containing TSP and (B) $CDCl_3$ containing TMS. Inserts (*) were recorded at 500-Hz sweep width. Peaks are identified in Figure 2, except for peak d which is assigned to the OH of Triton X-100. Please note that in the manuscript of Podo et al. (1973), the assignment of peaks h and i is reversed from our assignment (Dennis and Owens, 1973); a detailed discussion of the assignment appears elsewhere (Ribeiro and Dennis, 1975).

lene unit. In $CDCl_3$, peak e shows one main peak and a small shoulder on the low-field side of the main peak. Presumably, the shoulder reflects the same methylene group adjacent to the terminal hydroxyl group. Peaks f and g are resolved into clean triplets and h and i into an aa′bb′ pattern as shown in the insert in Figure 6B. The T_1 relaxation times of the various resonances in Triton X-100 in D_2O, $CDCl_3$, and CD_3OD are given in Table VII.

Discussion

Relaxation Times of Triton X-100 in Micelles and Mixed Micelles. The T_1 values differ for each class of pro-

Table III: ^{1}H NMR Parameters for Mixed Micelles.

		Micelles[a]		Mixed Micelles[b]				Multibilayers (Unsonicated)[c]			Vesicles (Sonicated)[d]		
		a	b	a	x	y	z	x	y	z	x	y	z
100 MHz	T_1 (sec)	0.23	0.098	0.23	0.50	0.36	0.43	0.26		0.29	0.42	0.32	0.38
	$\Delta\nu_{1/2}$ (Hz)	2.1	6.6	2.1	4.7		1.5	110 ± 20	1100	180 ± 50	17	34	~4
	T_2* (sec)	0.15	0.048	0.15	0.068		0.21						
	T_1/T_2*	1.5	2.0	1.5	7.3		1.9						
220 MHz	T_1 (sec)	0.27	0.12	0.26	0.68	0.43	0.51	0.33		0.37	0.50	0.37	0.29
	$\Delta\nu_{1/2}$ (Hz)	2.1	4.4	2.3	5.3		1.9		1700				
	T_2* (sec)	0.15	0.072	0.14	0.059		0.17						
	T_1/T_2*	1.9	1.7	1.9	11.4		3.0						
	T_2										0.036	0.056 (20%) <0.02 (80%)	0.075
	T_1/T_2										14	6.6 (20%) >19 (80%)	3.9

[a] 100 m*M* Triton X-100 at 34° (100 MHz) or 40° (220 MHz). [b] 100 m*M* Triton X-100 plus 33 m*M* dimyristoylphosphatidylcholine at 34° (100 MHz) or 40° (220 MHz). [c] Dimyristoylphosphatidylcholine at 30° (Feigenson and Chan, 1974). [d] 100-MHz data for dipalmitoylphosphatidylcholine at 40° for T_1 and 42° for $\Delta\nu_{1/2}$ (Lee et al., 1972). The line width for peak z was obtained from a figure in Lee et al. (1972). 220-MHz data for egg phosphatidylcholine at 20° (Horwitz et al., 1972). According to the authors, similar values were obtained for dimyristoylphosphatidylcholine, but data are not given (Horwitz et al., 1972, 1973).

Table IV: Concentration and Viscosity Dependence of Spin–Lattice Relaxation Times.[a]

	Triton		Triton + Phospholipid			
Peak	100 m*M*	400 m*M*	30 m*M*	50 m*M*	100 m*M*	400 m*M*
a	0.23	0.21	0.22	0.21	0.21	0.22
b	0.098	0.089	0.089	0.10	0.088	
c	0.096	0.094	0.10	0.11	0.095	
h	0.26	0.19	0.22	0.21	0.21	~0.19
	0.24	0.18	0.20	0.21	0.19	
i	0.19	0.18	0.19	0.21	0.21	~0.18
	0.18	0.18	0.18	0.18	0.21	
x			0.47	0.43	0.43	0.40
y			0.31	0.33	0.33	
z			0.40	0.41	0.43	0.40
Viscosity (P)	0.01[b]–0.02[c]	~0.8[d]				

[a] T_1 values (sec) at 34° for Triton X-100 containing dimyristoylphosphatidylcholine at a molar ratio of 3:1 Triton/phospholipid where indicated. See footnote *d* in Table II. [b] Calculated from the data of Kushner and Hubbard (1954) for 0.15–1.5 m*M* Triton X-100 and the data of Yedgar et al. (1974) for 3.2–80 m*M* Triton X-100 at 20°. [c] Calculated from the data of Greenwald and Brown (1954) for 150 m*M* Triton X-100 at 30°. [d] Calculated from the data of Greenwald and Brown (1954) for ~450 m*M* Triton X-100 at 30°.

Table V: Spin–Lattice Relaxation Times of Mixed Micelles in the Presence of Paramagnetic Mn^{2+}.[a]

Peak	None	0.05 m*M*	0.1 m*M*	0.5 m*M*	1 m*M*	5 m*M*
a	0.21	0.21	0.21	0.21	0.18	0.12
b	0.098	0.087		0.087	0.048	0.039
e						
1	0.42	0.35		0.35		
2	0.40	0.37		0.36		
3	0.42	0.34		0.33	0.15[b]	0.042[b]
4	0.68	0.31		0.38		
5	0.63	0.48		0.38		
6						
7	0.65	0.47		0.50		
h	0.21	0.18	0.19	0.21	0.14	0.075
	0.21	0.17	0.19	0.21		
i	0.19	0.17	0.20	0.18	0.14	0.072
	0.19	0.16	0.21	0.19		
x	0.45	0.41	0.41	0.43	0.27	0.13
y	0.33	0.30	0.29	0.34	0.20	0.13
z	0.40	0.24	0.18	0.15	0.055	Very Broad
HOD	>4.5[c]	1.05	0.66	0.56	0.059	Very Broad

[a] T_1 value (sec) at 34° for 100 m*M* Triton X-100 and 33 m*M* dimyristoylphosphatidylcholine titrated with $MnCl_2$. Sample was not degassed. See footnotes *b* and *d* in Table II. [b] Average value for peak e reported at high $MnCl_2$ since individual components were not resolved presumably because of the dominant paramagnetic effect of the high concentration of Mn^{2+}. [c] T_1 for HOD in absence of Mn^{2+} estimated from null. T_1 value at 40° for a sample degassed by freeze–pump–thaw cycles was 35.4 sec.

Table VI: Spin–Lattice Relaxation Times in H_2O/D_2O Mixtures.[a]

	Triton			Triton + Phospholipid[b]		
Peak	D_2O	5% H_2O	10% H_2O	D_2O	5% H_2O	25% H_2O
a	0.23	0.22	0.22	0.24	0.22	0.24
b	0.098	0.094	0.097	0.091	0.10	0.092
c	0.096	0.090	0.090	0.10		0.092
e						
1	0.37	0.34	0.34	0.38	0.32	0.35
2	0.37	0.37	0.35	0.43	0.38	0.33
3	0.42	0.40	0.44	0.43	0.43	0.32
4	0.52	0.49	0.48	0.49		0.50
5	0.93	0.60	0.83	0.80	0.67	0.64
6						
7	0.89	0.61	0.66	0.66	0.72	0.57
h	0.26	0.22	0.22	0.24	0.19	0.21
	0.24	0.22	0.22	0.23	0.19	0.19
i	0.19	0.20	0.17	0.21	0.18	0.19
	0.18	0.20	0.17	0.22	0.17	0.18
x				0.45	0.45	0.41
y				0.33	0.35	0.29
z				0.47	0.38	0.33

[a] T_1 values (sec) at 34° for 100 m*M* Triton X-100 containing 33 m*M* dimyristoylphosphatidylcholine where indicated. Note that 99.8% D_2O was employed in these experiments. See footnotes *b* and *d* in Table II. [b] T_1 values at several additional concentrations of H_2O up to 40% H_2O were obtained; for the sake of brevity, only three concentrations are shown. Above 5% H_2O content, these experiments necessitate adjustment of preamplifier gains to low and adjustments to obtain free induction decays that do not exceed the dynamic range of the Nicolet 1085 computer employed. Even so, at 40% H_2O, base lines skewed around the HOD peak. Fortunately, the hydrophobic peaks were chemically shifted far enough to allow T_1 determinations.

tons in the surfactant. The simplest interpretation to account for the various T_1 values is to assume that the main relaxation mechanism is dipole–dipole interaction between protons. In particular, if we consider only the contribution from the nearest neighbor protons that leads to intramolecular dipole–dipole interactions, then an equation for isotropic motions as discussed elsewhere (Farrar and Becker, 1971) may be applicable. This equation assumes that the T_1 values can be described by a single correlation time for motion, τ_c. In the so-called "extreme-narrowing limit" ($\omega\tau_c \ll 1$), longer T_1 values would suggest faster motions. This condition also predicts $T_1 = T_2$, that T_1 is frequency independent, and that log T_1 is proportional to temperature.

For the Triton micelles and mixed micelles, T_2* estimated from the line width gives ratios of T_1/T_2* of 1:2 for the Triton *tert*-butyl and dimethyl groups. These groups appear to be frequency independent and also exhibit a positive dependence of T_1 on temperature. If the motions of these groups were treated isotropically, a correlation time could be calculated; however, both these groups possess methyl rotors and may be more correctly described by anisotropic motional expressions with two or more correlation times. Woessner (1962) has shown that all of the observed phenomena would also occur for anisotropic motions of a methyl rotor on a reorienting axis in the extreme narrowing limit. Therefore, it can only be firmly concluded from the data that the *tert*-butyl and dimethyl groups are observed in the extreme narrowing limit.

If we consider the alkyl methylene and oxyethylene proton T_1 values in terms of isotropic motions, a short T_1 value for the alkyl methylene suggests less mobility in the hydrophobic alkyl chain than in the polar oxyethylene regions, and the trend in T_1 values for peaks g, f, and e suggests a mobility gradient may exist for the oxyethylene groups from the phenyl ring to the terminal oxyethylene unit. However, this interpretation may not follow directly because while these groups do show a positive dependence of T_1 on temperature, the T_1 values do appear to show a slight frequency dependence so that anisotropic motions are probably present.

If anisotropic motions are present, the apparent activation energies for the thermal relaxation processes (T_1) and

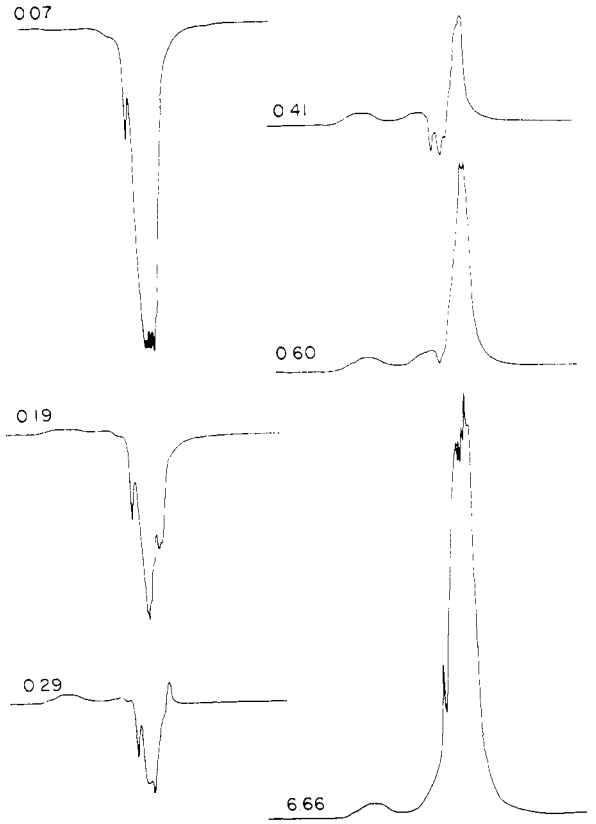

FIGURE 7: Partially relaxed spectra at the τ values specified (in sec) for the oxyethylene protons (designated peak e). The peak consists of several chemically shifted and partially overlapping lines with an asymmetry in height. The protons on the high-field side can be seen to relax before those on the low-field side.

the spin-exchange processes (T_2*) represent average values for the different motions of any one group. The values of 2–5 kcal/mol are reasonable for the occurrence of internal motions since they agree favorably with literature values for potential barriers to internal rotation in alkanes (Abe et al., 1966).

On the assumption that Triton micelles can be approximated as spheres (radius of about 48 Å) in a solution about the viscosity of water (0.01 P), Stokes law allows an estimate of 2×10^{-7} sec at 25° for the correlation time for micelle tumbling. This correlation time appears to be too long to contribute as a relaxation mechanism and if it did contribute it should be affected by solution viscosity. The T_1 values of the various groups in the Triton micelles appear to be independent of viscosity changes known to occur with concentration changes in aqueous solutions of Triton X-100. These results suggest that rotation of the micelle is not responsible for the relaxation times observed. The data are clearly supportive of a micelle model in which the molecules are not rigid but instead are highly mobile, and are consistent with the idea that the hydrophobic core of these nonionic surfactant micelles and mixed micelles have a liquid-like nature.

Anisotropic Phospholipid Motions in Mixed Micelles, Vesicles, and Multibilayers. Chan et al. (1973) have suggested that intramolecular dipole–dipole relaxation processes are dominant for phospholipids and have presented arguments against the dominance of vesicle tumbling (Finer et al., 1972b) or lateral diffusion (Metcalfe et al., 1973). Seiter and Chan (1973) considered the phospholipid motion to be anisotropic and using a modification of the treatment

Table VII: Spin–Lattice Relaxation Times of Triton X-100 in Various Solvents.[a]

Peak	D_2O[b]	CD_3OD[c]	$CDCl_3$[d]
a	0.23	1.1	1.1
b	0.098	0.50	0.59
c	0.096	0.63	0.73
d	Exchangeable		0.87
e			
1	0.37	1.4[e]	1.3[e]
2	0.37		
3	0.42		
4	0.52		
5	0.93		
6			
7	0.89	1.5	1.5
f	0.38	1.3	1.2
g	0.17	1.0	1.1
h	0.26	1.8	2.0
	0.24	1.7	1.8
i	0.19	1.1	1.1
	0.18	0.95	1.1

[a] T_1 values (sec) at 34°. See footnotes *b* and *d* in Table II. [b] 100 m*M*. [c] 330 m*M*. [d] 110 m*M*, sample contains 1% TMS. [e] Single sharp peak obtained with spike on downfield side designated 7.

of Woessner (1962) suggested the presence of two correlation times, $\tau_\parallel$ and $\tau_\perp$, for motions about a longitudinal chain axis. $\tau_\parallel$ is representative of a fast process and has been interpreted in terms of coupled pairs of trans–gauche rotations (especially B-coupled pairs). $\tau_\perp$ corresponds to slower chain motions that distort the chains from a linear profile (for example, uncoupled trans–gauche motions).

The degree of motional restriction on these anisotropic motions appears to be the most likely explanation for the similar T_1 values, but dissimilar line widths in the three different preparations of phospholipid. In the multibilayers, very tight packing of the phospholipid leads to great restrictions in $\tau_\perp$ resulting in lower apparent intensities while the fast motions $\tau_\parallel$ can occur. Thus, Seiter and Chan (1973) estimate that $\tau_\parallel \sim 10^{-9}$ sec and $\tau_\perp \sim 10^{-7}$ sec. For the sonicated vesicles, much looser packing is possible. Local motions can be less restricted and fast and full intensities are observed. Seiter and Chan (1973) estimate that $\tau_\perp < 5 \times 10^{-9}$ sec. However, significant anisotropy of the motion still exists. For vesicles, Horwitz et al. (1972, 1973) found experimentally that $T_2 < T_1$ and Mclaughlin et al. (1973) have conducted extensive temperature and frequency dependence studies; both groups concluded that two correlation times were necessary to describe the motion in vesicles.

Presumably intramolecular relaxation processes also account for the relaxation of the phospholipid in mixed micelles. The packing of the phospholipid in a mixed micelle where the surfactant has a rather short bulky hydrophobic group could be more disordered and looser than in a bilayer structure. Thus, one might expect $\tau_\perp$ to be even shorter. This is suggested experimentally by the fact that in the mixed micelle, the phospholipid molecule begins to show fine structure approaching that seen for phospholipids in organic solvents (Finer et al., 1972a; Metcalfe et al., 1973; Birdsall et al., 1972). In the hydrophobic region, the terminal methyl group appears as a clear triplet (Ribeiro and Dennis, 1975) (Fig. 2), whereas in the vesicle preparations broader lines are observed (Lee et al., 1972; Horwitz et al., 1973; Mclaughlin et al., 1973). T_1/T_2* for the methyl peak is about 7–11 while the data of Horwitz et al. (1972) for this group give T_1/T_2 of 14. Since T_2* is actually a lower

limit on T_2, the true T_1/T_2 ratio for the methyl group in the mixed micelles is presumably smaller than 7. Unfortunately, due to the overlap with the Triton dimethyl protons, we were unable to obtain a T_2^* value for the methylene protons. For the polar region, the choline methyl group in mixed micelles has a T_1/T_2^* ratio of 1.9–3.0 while Horwitz's data for T_1/T_2 give a value of 4 (Horwitz et al., 1972). It should be noted that in bilayer vesicles the choline peak actually consists of two peaks, resolvable at high magnetic fields or in the presence of paramagnetic ions (Bystrov et al., 1971; Kostelnik and Castellano, 1973; Levine et al., 1973), so that this situation may contribute to the broader line in vesicles.

Finally, we should note that the theory for dealing with relaxation phenomenon of phospholipids is not completely worked out and all interpretations must be made with caution. Nonetheless, the above considerations suggest that the T_1 values are ascribable to similar coupled gauche–trans-gauche isomerizations for phospholipid in multibilayers, vesicles, and mixed micelles. However, the magnitude of the anisotropic phospholipid motion varies for the three systems and the rate of local motion ($\tau_\perp$) presumably controls the line width (or T_2) in these systems. Thus, the degree of restriction on molecular motion appears to be even less in the mixed micelles than in vesicles.

Structure and Hydration of Micelles and Mixed Micelles. The micelles formed from alkylpolyoxyethylene and alkylarylpolyoxyethylene surfactants are thought to form spheres when the aggregate micelle molecular weights are about 100,000 and rods or disks when much larger (Schick et al., 1962). The Triton X-100 micelle has been suggested to be a hydrated sphere with molecular weight of 86,000–90,000 (about 143 monomers) (Kushner and Hubbard, 1954; Yedgar et al., 1974). These nonionic surfactant micelles are thought to have the hydrophobic groups located in the micelle interior and hidden from solvent by the polar oxyethylene groups which are exposed to solvent. Due to the higher magnetogyric ratio of the proton over the deuteron in causing effective intermolecular dipole–dipole interactions, T_1 values can be a sensitive parameter for probing micelle solvation since a shortened T_1 is expected upon going from D_2O to D_2O/H_2O mixtures for the micelle groups which are in contact with solvent. The consistency of the T_1 values for the Triton alkyl and phenyl protons within experimental error suggests a picture in which water does not penetrate the hydrophobic part of the Triton molecule and that these groups form part of an apolar cavity in the micelle interior.

Unfortunately, the chemically shifted oxyethylene protons which can be considered polar cannot be sufficiently resolved to serve as a control. We attempted T_1 measurements for these protons and found that some of the T_1 values do not appear to change significantly while some do. We are not sure whether this is due to a lack of resolution or due to a real process. Presumably, the changes in T_1 values of some of the oxyethylene units correspond to those groups which are exposed to solvent. Our results for the Triton X-100 molecule confirm the results of Podo et al. (1973) who recently reported the same type of experiment at 220 MHz for Triton micelles but our data extend this to mixed micelles. Clemett (1970) has reported that the T_1 values for the alkyl groups of *n*-decyl(tetraethoxy)ethanol micelles are independent of solvent H_2O or D_2O, while Corkill et al. (1969) on the basis of chemical shift measurements in micellar solutions of a series of alkyl (pentaethoxy)ethanols have reported that the amount of water penetrating the micellar core is independent of the alkyl chain lengths. Thus, it appears that water penetration of the hydrophobic core of all of these nonionic micelles does not occur.

The Mn^{2+} titration experiments are also consistent with this picture. At the lower concentrations of paramagnetic ions, where the T_1 value of the HOD resonance line representative of bulk solvent is decreased and the resonance line already broadened, the T_1 value of the Triton *tert*-butyl, dimethyl, and phenyl protons and the line width of the *tert*-butyl protons are essentially unchanged. This clearly suggests that these groups are secreted into the hydrophobic core and shielded from the paramagnetic effect of Mn^{2+}. On the other hand, the T_1 values of some of the oxyethylene components, particularly those at the low-field end of the band (see Figure 7), are decreased at the low concentrations of Mn^{2+}, consistent with the idea that these groups are exposed to bulk solvent. At high concentrations of Mn^{2+}, all of the T_1 values for the surfactant groups are decreased greatly; fine structure is no longer observed for the oxyethylene protons which appear to be characterized by a single T_1 value. Presumably, at those concentrations, the micelle surface and oxyethylene palisade layer binds Mn^{2+} ions at saturation levels, and the paramagnetic effects of Mn^{2+} relaxation become dominant.

It should be pointed out that the hydrophobic portion of Triton micelles need not be spherical. In fact simple geometrical considerations argue against this point since it appears unlikely that over 140 monomers of this surfactant which has a short bulky hydrocarbon portion can pack to give a spherical cavity, although the outer hydrophilic surface composed of the oxyethylene groups could give the overall morphology of a sphere. The exact configuration of the oxyethylene groups in the exterior of these nonionic surfactant micelles is unclear at this time. Rösch (1967) has discussed the "zig-zag" model and the "meander" model for the oxyethylene group, so that it could either be fully extended in the "palisade layer" on the exterior of the micelle, or else it could form helical coils which twist and wind in the palisade layer from the hydrophobic center to the micelle surface. The evidence for Triton X-100 micelles has been interpreted to favor the fully extended configuration of the oxyethylene groups, and that these micelles have a radius approximately that of the length of the monomer molecule, 43–48 Å (Kushner and Hubbard, 1954; Yedgar et al., 1974). The relaxation experiments reported here clearly cannot distinguish between the two configurations of the oxyethylene group.

The observation that the T_1 and T_2^* values for the surfactant molecule are not changed in the presence of phospholipid suggests that the presence of phospholipid in the mixed micelle does not affect the overall interactions present in the nonionic surfactant micelle. It may then be asked in what manner is this micelle capable of accommodating the relatively large phospholipid molecule into this structure? The simplest picture would be one in which the nonpolar side chains of the phospholipid are incorporated into the hydrophobic core of the nonionic micelle, while its polar groups are situated in the polar polyoxyethylene palisade layer and open to the aqueous environment as shown in Figure 8. The lack of change in the T_1 values for the phospholipid terminal methyl protons in the H_2O/D_2O experiments and at low concentrations of Mn^{2+} ions clearly suggest a hydrophobic environment for this group, presum-

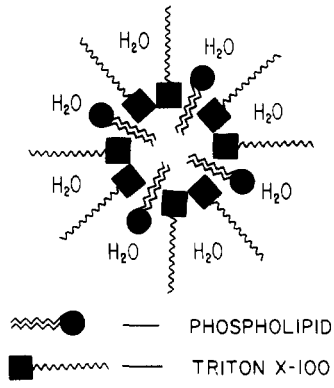

FIGURE 8: Schematic diagram for the structure of mixed micelles composed of Triton X-100 and phospholipid at a molar ratio of 2:1 Triton/phospholipid.

ably within the interior cavity of the micelle. The same kind of suggestion can be made for at least the bulk of the methylene protons of the fatty acid side chain. We can only detect an average T_1 value for these protons. Thus, we have no way of telling if water penetration might occur to the methylene protons adjacent to the carboxyl groups (C-2 protons). That this average T_1 value for these protons remains roughly constant in the presence of H_2O or at lower concentrations of Mn^{2+} is at least indicative that most of the methylene protons are hidden in the hydrophobic interior of the mixed micellar structure. By contrast, the polar choline methyl protons show an apparent decrease (admittedly small) in the T_1 value when solvent is changed from D_2O to mixtures of H_2O/D_2O and a much larger decrease in T_1 and increase in line width in the presence of Mn^{2+}. In fact, the line width broadening of this group is concomitant with the line width changes observed for the HOD peak which should be representative of bulk solvent (Figure 5). This is good evidence that the choline methyl protons are exposed to the aqueous environment, presumably somewhere in the polar palisade layer. Hence, upon mixed micelle formation, the phospholipid molecule appears to be merely intercalated into the Triton X-100 micelle in such a manner as to place its hydrophobic groups in the apolar core, and its polar groups into the polar exterior palisade layer.

Physical State of Triton in Various Solvents. Ray (1971) has recently proposed a scheme for considering the structures formed when amphiphilic molecules are dissolved in various solvents. In water and other polar solvents, "solvophobic interactions" are thought to bring about the formation of micelles, while no evidence for micelle formation in methanol and other "semipolar" solvents has yet been obtained (Ray, 1971; Kitahara et al., 1962; Fryar and Kaufman, 1969). Hydrocarbon solvents such as toluene and dodecane (Fryar and Kaufman, 1969) are thought to lead to the formation of "inverted" micelles (Becher, 1967). The magnitudes of the T_1 values for Triton X-100 presented in Table VII are consistent with Ray's scheme. The T_1 values for Triton X-100 are long in both chloroform and methanol, the latter solvent being one in which Ray could obtain no evidence for micelle formation by a nonylphenylpolyoxyethylene surfactant (Ray, 1971). By comparison, the T_1 values of all of the groups are much shorter in water than in either organic solvent. This is consistent with the micellar structure of Triton X-100 in aqueous solution discussed in this paper.

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Ion Binding by X-537A. Rates of Complexation of Ni^{2+} and Mn^{2+} in Methanol†

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ABSTRACT: The rates of complexation are studied through the effects of the paramagnetic ions upon the magnetic resonances of three of the proton species in X-537A = XH. For the dissociation of the complex $MX^+ \rightarrow M^{2+} + X^-$ at 25° the rate is $(2.4 \pm 0.4) \times 10^2$ sec^{-1} for Ni^{2+} and in the range from 2×10^4 to 1×10^6 sec^{-1} for Mn^{2+}. For the Ni^{2+} complex the activation parameters are also determined and discussed in terms of the details of the process. The difference in rate constants found here is much greater than the difference in the dissociation constants.

The work reported here is a continuation of an earlier study (Degani and Friedman, 1974) in which the stoichiometries, thermodynamics, and spectra of various complexes of M^+ and M^{2+} ions with the ionophorous antibiotic X-537A (Lasol acid) (Figure 1) were determined. For the reasons discussed in our previous study, the homogeneous solution chemistry of the antibiotic is of basic interest for the interpretation of the membrane phenomena in which the ionophore plays a role. This is as true for rates as for the properties already studied; however, very few rates of complexation of metal ions in solution have been determined for the cyclic ionophores (Diebler et al., 1969; Chock, 1972; Funck et al., 1972) and, until now, none at all for the carboxylic ionophores.

Here the kinetics of the Mn^{2+} and Ni^{2+} complexes in methanol are reported as determined by the effect of the complexation process upon the magnetic resonance of various protons in the antibiotic. While the use of paramagnetic metal ions is dictated by the method chosen here, one's interest in the interaction of X-537A with these ions may be enhanced by the report that in the presence of X-537A the M^{2+} ions all have similar effects upon nerve synapses (Kita and Van Der Kloot, 1974).

As in our previous report (Degani and Friedman, 1974) we write XH for X-537A and $XH \rightarrow X^- + H^+$ for the reaction in which it ionizes. Reference may be made to the earlier study for the determination of the ionization constant of XH in methanol as well as for the equilibrium constants of the reactions:

$$M^{2+} + X^- \rightarrow MX^+$$

which are required in the present study. The latter results are summarized in Table I. They were determined from ex-

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