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Effect of Micellar Interactions on Base-Catalyzed Hydrolysis of Procaine[1]

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The modifying effects of polyoxyethylene lauryl ether, sodium lauryl sulfate, cetyltrimethylammonium bromide and N-dodecyl betaine micelles on the rate of base-catalyzed hydrolysis of procaine were investigated. The retardation effect for the hydrolysis was observed in any surfactant solutions. Partition coefficients (K) to the micelles were calculated from k_{obs} and the partial molar volume based on the phase separation model. The order of K was found to be SLS>CTAB>NDB>PLE for the free base.

In the cationic surfactant system, the corresponding p-nitro substituted of procaine was also retarded in hydrolysis rate. These observations are contrary to the electrostatic theory. It could be considered that the effect of diethylaminoethyl moiety rather than p-substituents was predominant in the solubilization mechanism.

The determination of partition coefficients by the potentiometric method was made for PLE and CTAB systems and a fairly good agreement with the values from kinetics data was obtained.

Nuclear magnetic resonance studies suggested that in the case of SLS and PLE the solubilization of procaine was related as far as the center portion of the micelle whereas the drug molecule was located at the outer layer for CTAB and NDB micelles.

Keywords——base-catalyzed hydrolysis of procaine; micellar interaction; polyoxyethylene lauryl ether; sodium lauryl sulfate; N-dodecyl betaine; cetyltrimethylammonium bromide; 2-diethylaminoethyl p-nitrobenzoate; potentiometric titration; NMR spectroscopy for micellar interaction; partition coefficient to micellar phase

The modifying effect of surfactants on the rate of organic reactions has been studied, in which specifically their influence on drug stability has been paid attention in the field of pharmaceutical preparation.[3–5] A variety of micellar catalyses were comprehensively surveyed by Fendler and Fendler.[6] The effect of surfactants on the kinetics of organic reaction principally results from the fact that the drug is partly associated with micelles which give a different environment from the bulk phase for the reaction site.

In the base-catalyzed hydrolysis of relatively simple aromatic esters, anionic, nonionic and zwitterionic surfactants cause a rate retardation for the ester cleavage.[7,8] Meanwhile, it is suggested that the effect of cetyltrimethylammonium bromide was related to the p-substituent of the aromatic ring, the surface pH of the micelle and the dielectric property at the surface of the micelle.[9] With regard to the p-substituent, the hydrolysis rates of ethyl p-nitrobenzoate and p-nitrophenylacetate were enhanced by cetyltrimethylammonium bromide whereas those of their corresponding derivatives were retarded.[10]

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3) P.B. Sheth and E.L. Parrott, *J. Pharm. Sci.*, **56**, 983 (1967).
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It has been shown that procaine was subject to hydrolytic degradation, yielding *p*-aminobenzoic acid and diethyl ethanolamine.[11] The reaction was due to the base-catalyzed hydrolysis of the ester linkage. The present study was undertaken to assess the influence of various surfactants with different nature on the hydrolysis rate of procaine based on the partition model which includes the partition coefficient between the micellar phase and the bulk aqueous phase and the reaction rates of the solubilized and unsolubilized species. This report also concerns the effect of cetyltrimethylammonium bromide on the hydrolysis rate of the corresponding *p*-nitro substituted of procaine and possible manners of the micellar interactions.

Experimental

Materials——Procaine HCl, J.P. ($pK_a = 8.8$ at 40°) obtained commercially was recrystallized from EtOH. 2-Diethylaminoethyl *p*-nitrobenzoate was synthesized in the manner that an equimolar mixture of *p*-nitrobenzoic acid and diethyl-(2-chloroethyl)amine was refluxed in isopropyl alcohol on a steam bath. The product was recrystallized from isopropyl alcohol (yield, 70%), mp 174—175°. Polyoxyethylene lauryl ether (PLE) was purified from commercially available Brij 35 as previously mentioned.[12] Sodium lauryl sulfate (SLS) was purified from Texapon L 100, Henkel Co., by recrystallization from *n*-BuOH after extraction of impurities with ether for 35 hours by a Soxhlet apparatus. Cetyltrimethylammonium bromide (CTAB) obtained commercially was recrystallized from CCl_4. N-Dodecyl betaine (NDB) was synthesized by the method of Bechett and Woodward and recrystallized from EtOH–ether.[13] The critical micelle concentrations for PLE, SLS, CTAB and NDB at 25° are 9.1×10^{-5}, 8.1×10^{-3}, 9.2×10^{-4} and 1.8×10^{-3} M, respectively.[6] D_2O and tetramethylsilane (TMS) for nuclear magnetic resonance (NMR) study were of Merck products.

Kinetic Studies——Kinetic studies were carried out over pH range 7.0—12.5. Buffer solutions used as reaction media for hydrolysis are 0.05 M NaH_2PO_4–Na_2HPO_4 system at pH 7.0—8.0, 0.1 M NH_4Cl–NH_4OH at pH 8.0—10.5 and 0.05 M Na_2HPO_4–Na_3PO_4 at pH 10.5—12.5. The ionic strength was adjusted 0.2 by adding NaCl throughout. For each hydrolysis study with a particular surfactant the initial concentration of procaine was maintained 2.0×10^{-3} M. The surfactant concentration was ranged from 0 to 3.33×10^{-2} M. At an appropriate interval a 2-ml aliquot was withdrawn and diluted with pH 9.5 buffer. A residual procaine was assayed at 287 nm by a Hitachi 124 spectrophotometer. No influence on the absorption spectrum was observed in the diluted condition for any surfactant systems.

Potentiometric Titrations——The titration was carried out in a double-jacketed beaker equipped with thermostated water circulation by a TOA HS 2A pH stat. 20 ml of 6.0×10^{-3} M procaine solution and 10 ml of surfactant solution with varying concentrations were mixed in the beaker. Immediately after the solution was titrated with 6.0×10^{-2} N NaOH. The titration finished within 30 seconds. NaOH solution and the titration beaker were held under nitrogen stream.

NMR Measurements——The NMR spectra were obtained using a JEOL PS-100 NMR spectrometer operating at 24±1° and with TMS as an external reference. Chemical shifts were measured in ppm unit from TMS signal with an accuracy of ±0.01 ppm.

Determination of the Partial Molar Volumes——The density of the surfactant solutions was determined with a Lipkin-Davison type pycnometer. The calculated partial molar volumes of surfactants at 40° and pH 11.8 were 1110, 255, 378 and 319 ml/mol for PLE, SLS, CTAB and NDB, respectively. The pH dependency of the partial molar volume was little over pH 2.0—12.5 range for each surfactant.

Results and Discussion

The base-catalyzed hydrolysis of procaine was examined in the solutions of PLE, NDB, CTAB and SLS surfactants. The reaction followed the first order kinetics with regard to the ester concentration in any surfactant solutions. The concentration of a particular surfactant was varied as mentioned above, in which the lowest concentration of the surfactant was always more than each critical micelle concentration. As shown in Table I, the retardation effect for the hydrolysis was observed in any surfactant solutions as the surfactant

11) T. Higuchi, A. Havinga, and L.W. Busse, *J. Amer. Pharm. Ass., Sci. Ed.*, **39**, 405 (1950).
12) K. Ikeda, K. Kato, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2510 (1971).
13) A.H. Beckett and R.J. Woodward, *J. Pharm. Pharmacol.*, **15**, 422 (1963).

concentration increased. These are typical examples of the retardation effect for the hydrolysis conducted at pH 11.8 and 7.0.

Table I. The Obserbed Rate Constants for Hydrolysis of Procaine in Various Surfactant Solutions

PLE (10^{-2} M)	$k_{obs}\times10^3$ (min^{-1}, pH=11.8)	SLS (10^{-2} M)	$k_{obs}\times10^3$ (min^{-1}, pH=11.8)	$k_{obs}\times10^6$ (min^{-1}, pH=7.0)	CTAB (10^{-2} M)	$k_{obs}\times10^3$ (min^{-1}, pH=11.8)	NDB (10^{-2} M)	$k_{obs}\times10^3$ (min^{-1}, pH=11.8)
0.00	9.58	0.00	9.58	44.5	0.40	5.83	0.20	7.83
0.83	4.74	1.00	3.32	12.5	0.70	4.40	0.67	5.53
1.67	3.02	1.50	2.35	9.25	1.00	3.55	1.33	3.98
2.50	2.25	2.00	2.00	7.48	2.00	2.24	2.00	3.02
3.33	1.68	2.50	1.62	5.45	2.50	1.94	2.66	2.35
		3.00	1.30		3.33	1.52	3.33	2.03
		3.33	1.08	3.15				

The rate-pH profiles in respective surfactant solutions were investigated at the surfactant concentration of 3.33×10^{-2} M (Fig. 1). In the region of pH$>$pK_a=8.8, where procaine takes to greater extent an electrically neutral form, relatively large retardation effect on the hydrolysis was observed in every surfactant solution and the pH dependency showed the similar pattern in appearance.

The order of a retardation effect of micellar solutions was found to be SLS$>$CTAB$>$PLE$>$NDB which suggests the differences in reactivity of the drug in the micellar phase and in the bulk phase and/or the differences of the partitioning behavior of the drug between these two phases. As pH decreases, the effect became minor except for the SLS case, converging toward the rate of the nonsurfactant case. At pH=7.0, where the protonated form is predominant, the rates fell in the same value within an experimental error, although only SLS retarded about 10 fold slower. The results indicate that the procaine molecule, whether it is either free base or protonated, is associated with SLS micelles whereas only free base with PLE, CTAB and NDB micelles.

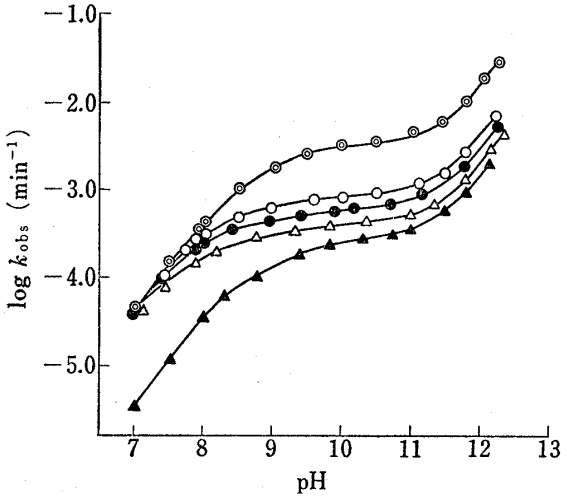

Fig. 1. The Rate–pH Profiles of Procaine Hydrolysis in Nonmicellar (◎), NDB (○), PLE (●), CTAB (△) and SLS (▲) Solutions at 40°

The surfactant concentration is 3.33×10^{-2} M throughout.

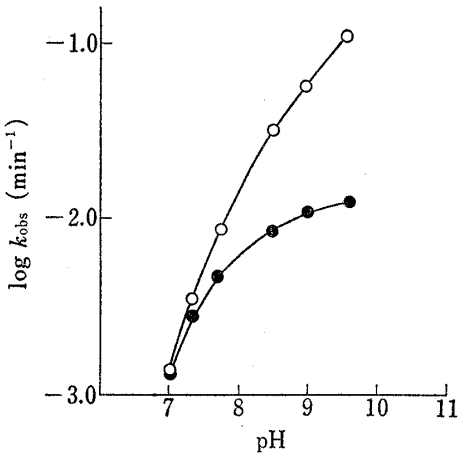

Fig. 2. The Rate–pH Profiles of 2-Diethylaminoethyl *p*-nitrobenzoate in Nonmicellar (○) and Cetyltrimethylammonium Bromide Solution (●, 3.33×10^{-2} M) at 25°

According to the electrostatic theory,[14] the base-catalyzed hydrolysis would be enhanced by cationic micelles and retarded by anionic ones. The retardation of the procaine hydrolysis with CTAB micelles is clearly contrary to the theory. Meakin and others reported that the presence of CTAB micelles increases the rate of ethyl *p*-nitrobenzoate and *p*-nitrophenylacetate but decreases that of ethyl *p*-aminobenzoate and *p*-aminophenylacetate.[10] The opposite effect was explained by the difference of the inductive effect of *p*-substituents, an electron-withdrawing group of $-NO_2$ and an electron-releasing group of $-NH_2$. From UV spectral study,[8] it was expected that ethyl *p*-aminobenzoate is oriented near the exterior of CTAB micelles. Eriksson and Gilberg suggested from NMR measurements that simple aromatic compounds like N,N-dimethylaniline and nitrobenzene were located at the micellar surface for electrostatic interactions between the solubilizate and CTAB micelles.[15] Accordingly, it is thought here that the hydrolysis rate for simple aromatic esters depends on the orientation manner of the solubilizate on the micellar surface.

Meantime, to compare the effect of *p*-nitro substituent with *p*-amino group of procaine on the rate of hydrolysis, 2-diethylaminoethyl *p*-nitrobenzoate (I) was examined in the same manner. As the rate of I was much faster than that of procaine, the temperature was lowered to 25°. In Fig. 2 is shown the rate-pH profiles of I in the CTAB concentration of 3.33×10^{-2} M and in the nonmicellar solution. As observed in the case of procaine, CTAB micelles also exerted a retardation effect for the base-catalyzed hydrolysis of I, which means the NO_2 substituent did not cause a rate enhancement and the case was also contrary to the theory.

In contrast with relatively simple aromatic esters, the present results would be expected if the diethylamino ethanol moiety rather than *p*-substituents was involved in the solubilization mechanism: the site of ester cleavage is adsorbed on the micellar surface which consists of the cationic head group of the surfactant or is located in the hydrophobic interior of CTAB micelles.

In quantitative analysis of micellar interactions the phase separation approach was taken in which the micelle occupies a part of the total volume of the system and the drug is able to partition into the micellar phase.[16]

When the drug partitions between the micellar and the bulk phases and undergoes the first order hydrolysis, the observed rate constant, k_{obs}, may be expressed as follows:

$$k_{obs} = k_w(\text{fraction in the bulk phase}) + k_m(\text{fraction in the micellar phase})$$

$$= \frac{1-v}{1-v+Kv} k_w + \frac{Kv}{1-v+Kv} k_m \quad (1)$$

where k_w and k_m are the rate constants of procaine in the bulk phase and in the micellar phase, respectively. v is the volume fraction of the micellar phase and K the partition coefficient, defined by

$$K = \frac{(Pr)_m}{(Pr)_w} \quad (2)$$

where $(Pr)_m$ and $(Pr)_w$ are the concentrations of the partitioned and unpartitioned procaine, respectively.

Assuming that the rate of hydrolysis is negligible in the micellar phase, the second term of equation 1 could be neglected. However, manipulating equation 1 for the general applicability, which covers such case that there might be an appreciable rate of hydrolysis in the micellar phase, one writes

$$k_{obs} - k_w = \frac{1}{K} \cdot \frac{(k_w - k_{obs})(1-v)}{v} + k_m - k_w \quad (3)$$

14) G.S. Hartley, *Trans. Faraday Soc.*, **30**, 444 (1934).
15) J.C. Eriksson and G. Gilberg, *Acta Chem. Scand.*, **20**, 2019 (1966).
16) A.G. Michel and J.F. Broadhead, *J. Pharm. Sci.*, **56**, 1261 (1967).

The linear expression of equation 3 yields the slope of $1/K$ and the intercept of $k_m - k_w$ when k_w, k_{obs} and v have been experimentally determined, and consequently K and k_m can be assessed.

The applicability of equation 3 was tested using k_{obs} values determined in the SLS, PLE, NDB and CTAB solutions and respective v values. The plot of the procaine hydrolysis data at pH=11.8 and 7.0 were shown in Fig. 3 and 4, in which the surfactant concentration decreases as the abscissa goes rightward. The linearity was satisfactorily established for both surfactant solutions and pH studied. As can be seen in Fig. 3, it should be noted that the extrapolation of the straight lines toward the ordinate converged to a value of -9.5×10^{-3} at the intercept, which was almost equal to the normal reading of k_w at pH=11.8. This implies that the rate of procaine hydrolysis was extremely slow or negligible in the micellar phase of any surfactant solutions examined, as equation 3 predicted. At pH=7.0 (Fig. 4), the plot was made only for the SLS case since little difference was recognized between the values of k_{obs} in the presence of other surfactants and k_w. Similarly, the hydrolysis rate of the drug in the SLS micelles was negligible because of $|k_{obs} - k_w| \simeq k_w$.

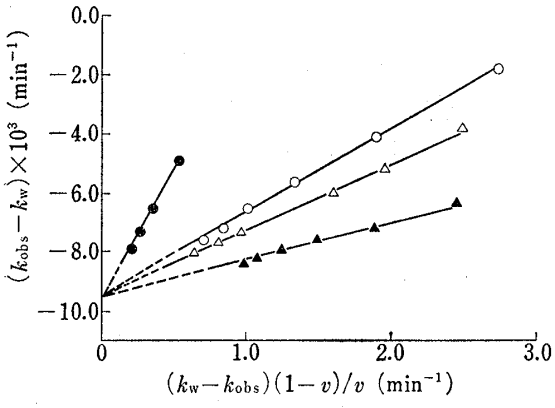

Fig. 3. Plots of $(k_{obs}-k_w)$ against (k_w-k_{obs}) $(1-v)/v$ at pH 11.8 and 40°

The surfactant concentration decreases as the abscissa goes rightward.
●, PLE; ○, NDB; △, CTAB; ▲, SLS.

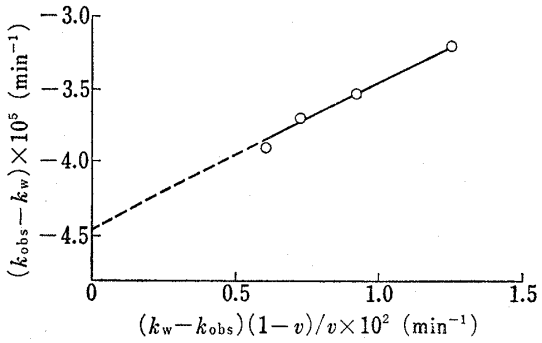

Fig. 4. Plots of $(k_{obs}-k_w)$ against (k_w-k_{obs}) $(1-v)/v$ for SLS System at 40° and pH 7.0

The surfactant concentration decreases as the abscissa goes rightward.

The partition coefficients estimated from the slopes were summarized in Table II. When the rate of hydrolysis in the micellar phase is negligible, the magnitude of k_{obs} is simply proportional to the fraction of the drug in the bulk phase which determines the partition coefficient. The order of the partition coefficient of free base was found to be SLS>CTAB>NDB>PLE, and did not correlate with that of the hydrolysis retardation effect regarding the order of PLE and NDB. This is because the observed rate constants were not obtained

Table II. Partition Coefficients Obtained by Kinetics Analysis and Potentiometric Titration at 40°

Surfactant	Kinetics		Potentiometric titration
	Protonated[a]	Free base[b]	Free base
PLE	0	104	118
CTAB	0	437	508
NDB	0	350	—[c]
SLS	952	690	—[d]

a) Determined at pH 7.0.
b) Determined at pH 11.8.
c) Could not be determined.
d) Titration curve showed no appreciable change.

under the condition of an identical volume fraction of the micellar phase but at the identical surfactant concentration (3.33×10^{-2} M).

It is clearly shown that there is a great contrast of the micellar interactions between the free base of procaine and its protonated form. The polarity of the diethylamino moiety would play an important role in interacting with micelles. Relatively large partition coefficient of the protonated form to the SLS micelles is compatible with the electrostatic theory and complete expulsion takes place for the CTAB, NDB and PLE micelles due probably to the diethylamino moiety positively charged at pH=7.0. Regarding the free base of procaine, the micelles with polar head group, whether it is anionic, cationic and zwitterionic surfactants, apparently attract more the molecule onto or into the micellar surface, leading to greater partition coefficients than that to the nonionic surfactant. It appears that the interaction between the head group of micelles and the intramolecular polarization of the procaine molecule is a primary mechanism for the partitioning.

An attempt was made to estimate the partition coefficient to the micellar phase using the potentiometric titration method. The method has been employed in nonionic and anionic surfactants[17] and is only applicable to such case that the amount of drug degraded can be ignored during a titration run. The procaine hydrolysis rate was slow enough to tolerate this method.

The relationship between the hydrogen ion concentration and the titration ratio, N, can be represented by

$$(H^+)_1 = K_a \cdot \frac{1-N}{N} \tag{4}$$

and

$$(H^+)_2 = K_a \cdot \frac{1-N}{N} \cdot \frac{1-v+Kv}{1-v} \tag{5}$$

where subscript 1 and 2 refer to the absence and presence of surfactant. When the titration ratio is identical between two conditions, one writes

$$\frac{(H^+)_2}{(H^+)_1} = 1 + \frac{Kv}{1-v} \tag{6}$$

Equation 6 indicates that the value of K can be determined from the slope of the plot of $(H^+)_2/(H^+)_1$ against $v/1-v$. Fig. 5 is one example of the titration curves with varying concentration of PLE. Fig. 6 illustrates the linearity of the plots determined at each half neutralization point in the solubilization by PLE and CTAB. As shown in Table II, the agreement of the values determined by two different methods is fairly good. In the SLS system, however, an appreciable shift of the titration curve was not observed due probably to the relatively close partition coefficient of the protonated form and the free one. In the presence of NDB the curves appeared unorganized with regard to the NDB concentration increment. This seems due to the interference of weak acidic group of the surfactant.

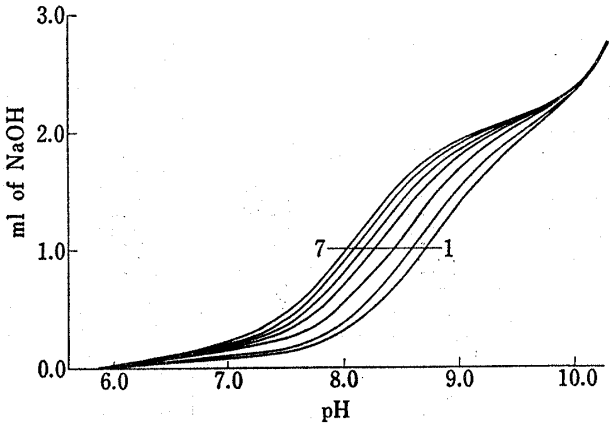

Fig. 5. Potentiometric Titration Curves of Procaine Hydrochloride (4.0×10^{-3} M) with NaOH (0.06 N, f=0.998) at 40°

Concentration of PLE (mM): 1) 0.00; 2) 4.17; 3) 8.34; 4) 16.7; 5) 25.0; 6) 33.3; 7) 41.7.

17) C.T. Rhodes and M. Donbrow, *J. Pharm. Sci.*, **54**, 1069 (1965).

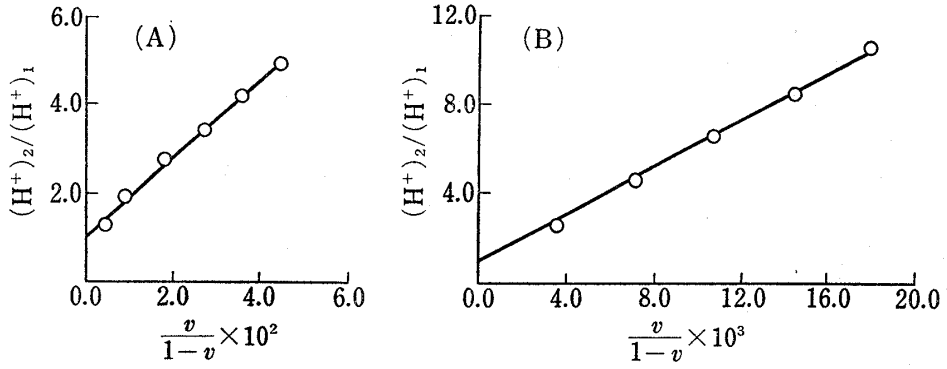

Fig. 6. Plots of $(H^+)_2/(H^+)_1$ against $v/1\text{-}v$ According to Equation (6)
(A), in PLE system. (B), in CTAB system.

NMR spectroscopic measurements have provided useful informations about the positional relation between the solubilizate and micelles.[18,19] The chemical shifts of hydrogens at different positions in the surfactant molecule were measured in the presence and absence of procaine. The concentration of procaine was maintained 0.073 M for the surfactant solutions examined.

In Table III is listed the degree of shifting for hydrogens of various part of the surfactant. As can be seen, the resonance lines generally shifted toward higher applied field. This is consistent with the well-known fact that the aromatic ring usually causes a higher magnetic shift for the hydrogen of a neighbouring molecules and such a shift is also observed by a change of medium for the objective molecule from a polarizing to a more hydrophobic environment.

Table III. ^{1}H Chemical Shifts for Surfactants in the Presence and Absence of Procaine in 0.1 N NaOD[a,b]

Procaine (M)	PLE			SLS			CTAB		
	$(C\underline{H}_2C\underline{H}_2O)_n$	$(C\underline{H}_2)$	$C\underline{H}_3(CH_2)_n$	$C\underline{H}_2SO_4^-$	$(C\underline{H}_2)$	$C\underline{H}_3(CH_2)_n$	$\overset{+}{N}(C\underline{H}_3)_3$	$(C\underline{H}_2)$	$C\underline{H}_3(CH_2)_n$
0	3.74	1.33	0.92	4.04	1.31	0.89	3.20	1.31	0.88
0.073	3.71	1.25	0.86	3.99	1.19	0.81	3.07	1.28	0.89
$\Delta\delta$	+0.03	+0.08	+0.06	+0.05	+0.12	+0.08	+0.13	+0.03	−0.01

Procaine (M)	NDB				
	$C\underline{H}_2COO^-$	$C\underline{H}_2(CH_2)_nCH_3$	$\overset{+}{N}(C\underline{H}_3)_2$	$(C\underline{H}_2)_n$	$C\underline{H}_3(CH_2)_n$
0	3.85	3.56	3.22	1.30	0.87
0.073	3.81	3.47	3.18	1.25	0.87
$\Delta\delta$	+0.04	+0.09	+0.04	+0.05	±0.00

a) In parts per million obtained at 100 MHz and 24±1° relative to external TMS.
b) The concentration of surfactant is 40 mg/ml throughout, *i.e.*, PLE, 0.033 M; SLS, 0.193 M; CTAB, 0.110 M; NDB, 0.130 M. These concentrations are above their critical micelle concentrations. (See experimental section)

For PLE and SLS the addition of procaine brought appreciable shifts of $-CH_2-$ and CH_3-C hydrogen resonance lines. The results could be explained by what the solubilization of procaine is related as far as the location of the CH_3-C part assumed to be very interior of the micelle.

In the case of CTAB and NDB somewhat less shifts of interior hydrogens were observed, in which especially the CH_3-C hydrogen resonances are scarecely affected, demonstrating that the drug is located at the outer layer of the micelle. The $N^+(CH_3)_3$ group of the CTAB micelles, however, assumed to be the most predominant site for the procaine solubilization.

18) J.H. Fendler, E.J. Fendler, G.A. Infante, Pong-Su Shih and L.K. Patterson, *J. Am. Chem. Soc.*, **97**, 89 (1975).
19) J.E. Gordon, J.C. Robertson, and R.L. Thorne, *J. Phys. Chem.*, 74, 957 (1970).