Arch. Pharm. Res. 9(3), 139-144 (1986)

Fluorescence Probe Study on the Solubilization Sites of Aniline Derivatives in Triton X-100 and Zephiramine Micelles

Suk Kyu Han and Yong Soo Lee

College of Pharmacy, Pusan National University, Pusan 607, Korea
(Received June 25, 1986)

Abstract □ The solubilization sites of aniline and its derivatives in micelles were investigated with fluorescence probe technique. The fluorescence probes employed in this study are 12-(9-anthroyl) stearic acid (AS) and 2-p-toluidinylnaphthalene-6-sulfonate (TNS) which are incorporated in the interior of the micelle and attached to its surface, respectively. As these two probes were effectively quenched by aniline and its derivatives, the modified Stern-Volmer relationship in micellar system could be applicable to estimate the partition coefficient, K_P of the solubilizate between aqueous and micellar phase. Because K_P derived by this method reflects the relative proximity of the fluorophore to the quencher, the ratio of K_P in the surface area to that in the interior of the micelle is interpreted in terms of the relative location of the solubilizate in micellar aggregate. The results show that the solubilizates are not located in a definite position but distributed in the multiple-sites of the micelle. The solubilization sites of the solubilizates in the micelle are dependent on their structures. As the solubilizate has more numbers of N-substituents of aniline and more numbers of carbon in the substituent, it tends to incorporate in the interior of the micelle more effectively.

Keywords □ Solubilization site, Fluorescence probe technique, Stern-Volmer relationship, Partition coefficient, Aniline derivatives, Triton X-100, Zephiramine.

Aqueous surfactant solutions continue to attract a reat deal of current attention. A surfactant micelle rovides a microcosm in which many interesting ıemical phenomena occur. Various micelles have ɛen used to solubilize a wide variety of water-in-)luble molecules and also to catalize many reac-on which are tedious in homogeneous solutions l). In order to elucidate the nature of micellar ffects on the properties of solubilized molecules,) understand the reaction mechanism in micellar stems and to design the micellar systems which anifest the desired properties, it is essential to ıow the solubilization sites of molecules. Many tempts have been made to study the solubilization te by X-ray diffraction methods, NMR methods,)sorption spectroscopy, ESR methods, fluores-nce depolarization methods, and others (2-5). owever, the solubilization sites in micellar sys-m are not yet satisfactorily understood.

The physical model usually presented to describe e solubilization behavior of micelles is one where e solubilizate is either incorporated in the interior of the micelle or attached to its surface, or both (6). The position of the solubilized additive within the micelle is considered to depend on the relative hydrophobic or hydrophilic nature of the substrate. Mukerjee and Cardinal (7, 8), using the sensitivity to polarity of the ultraviolet absorption characteristics of benzene and a number of its derivatives, interpreted their solution behaviors in the aqueous micelle of sodium lauryl sulfate (NaLS) in terms of a "two-state" model. In their model, solubilized species were considered to be distributed between a nonpolar "dissolved" state associated with the hydrocarbon core and a polar "adsorbed" state associated with the micelle-water interface. However, there has been little further experimental support for this proposal and the possibility of multiple-site occupancy of molecules remains a contentious issue.

In recent years the fluorescence probe technique has been extensively used as a means of investigating micellar properties such as viscosity and polarity of micellar microenvironment (9). The

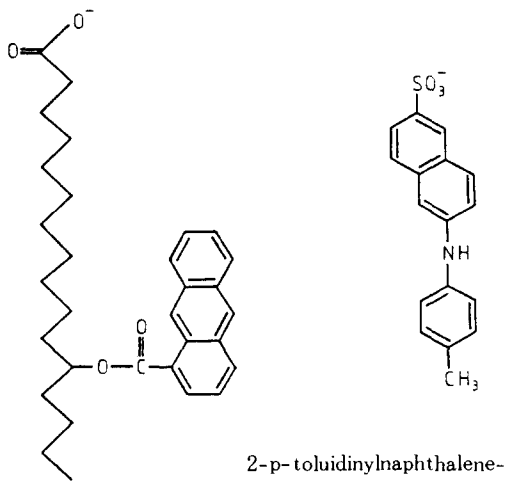
photoluminescence methods have also been used to obtain information on the solubilized sites of molecules by comparison of fluorescence properties (spectral shift, quantum yield, lifetime, depolarization, etc.) of micellized molecules with those of molecules in aqueous and hydrocarbon solutions (10) or kinetic analysis of fluorescence quenching of solubilized molecules by quenchers which are thought to be completely micellized (11) or stay exclusively in the aqueous phase (12). However, very few attempts have been made to determine the relative solubilization site of molecules by the use of partition coefficients which may be derived from quenching data.

In this study, the degrees of quenching of 12-(9-anthroyl) stearic acid (AS) and potassium 2-p-toluidinylnaphthalene-6-sulfonate (TNS) by aniline and its derivatives in nonionic and cationic surfactant micelles were measured and analyzed. AS and TNS are fluorescence probes which are known to incorporate into the core and the surface of micelles, respectively (13, 14). Aniline and its derivatives were selected for substrate because they effectively quench the fluorescence of these probes (15).

EXPERIMENTAL METHODS

Materials

The fluorescence probes (Fig.1), 12-(9-anthroyl) stearic acid and potassium 2-p-toluidinylnaphthalene-6-sulfonate were purchased from Sigma Chemical Co. (USA), and used without further purification. Triton X-100, a nonionic surfactan was a commercial, polydisperse preparation of -(1, 1, 3, 3, -tetramethylbutyl) phenoxypol -oxyethyleneglycols, containing an average of 9 5 oxyethylene units per molecule. Zephiramine, cationic surfactant was tetradecyl-dimethylbenzy ammonium chloride. These two surfactants wer supplied from Wako Pure Chemical Industry C (Japan) and used as received. Aniline, N-metl ylaniline and N,N-dimethylaniline were purchase from Hayashi Pure Chemical Co. and N,N-dietl ylaniline was supplied from Junsei Chemical Co. A other reagents were reagent grades, and water w double-distilled.



12-(9-anthroyl) stearic acid — 2-p-toluidinylnaphthalene-6-sulfonate

Fig.1. Chemical structure of the fluorescence probes.

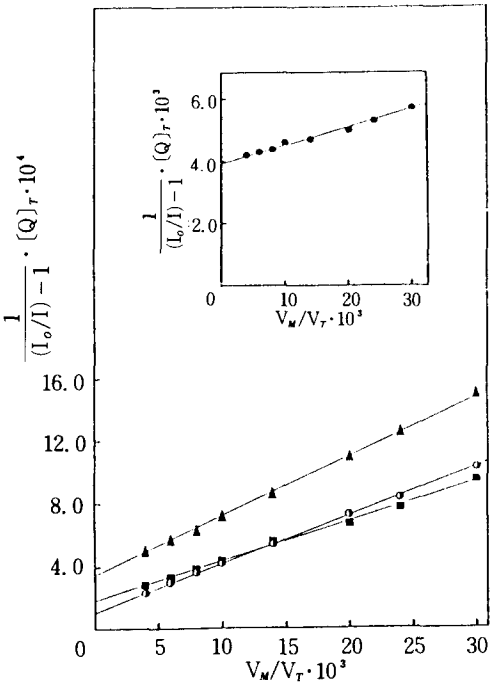


Fig.2. Quenching of AS in Triton X-100 micel lar solution by Aniline(●), N-meth ylaniline(■), N,N-dimethylaniline(▲) and N,N-diethylaniline(◑) at roon temperature plotted according to eq.(6)

Fluorescence Measurement

Surfactant micelles were labeled by addition microliter quantities of a concentrated probe sol tion in ethanol (for AS) or in dimethylsulfoxide (f TNS) and incubating 1 hour in the dark at roo temperature. Ethanol concentrations did not exce 0.2% and dimethylsulfoxide concentrations d not 0.7% in the final solutions. The final conce trations of AS and TNS were 5×10^{-6} M. The flu rescence intensities of AS or TNS were measured the absence and in the presence of a solubiliz

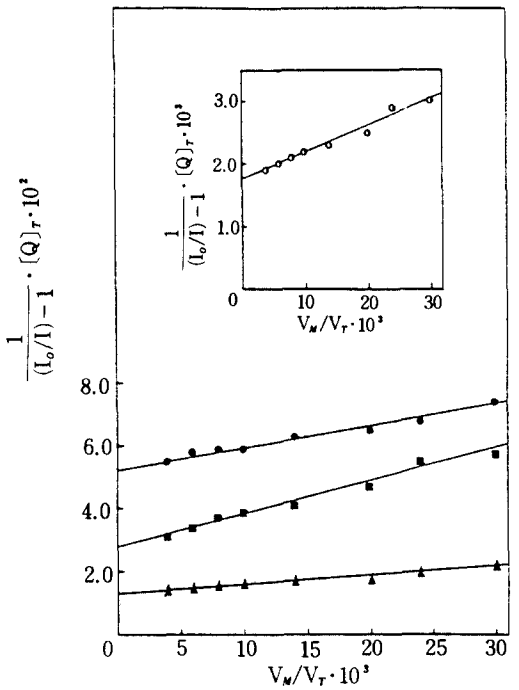


Fig.3. Quenching of TNS in Triton X-100 micellar solution by Aniline(●), N-methylaniline(■), N,N-dimethylaniline(▲), and N,N-diethylaniline (◑) at room temperature plotted according to eq.(6).

with Perkin-Elmer(LS-5) Luminescence Spectrometer. The fluorescence probe, AS was excited at 386nm and its emisson recorded at 458 nm. Excitation of TNS was at 370nm and emission was read at 455nm. The spectral bandwidths for excitation and emission were kept at 5nm. All experiments were carried out at least three times, and each point on a graph represents the average of these experiments. The relative fluorescence values from experiment to experiment did not differ by more than ±4%. All measurements were taken at room temperature.

RESULTS AND DISCUSSION

The partition coefficient of solubilizate between aqueous and micellar phase could be obtained from the quenching data by a method described in detail previously for lipid bilayers (16) and briefly outlined below. The dynamic quenching of a fluorescent molecule in free solution is described by the Stern-Volmer relationship:

$$I_o/I-1=K_{SV}(Q)_T=\tau_F k_q(Q)_T \qquad (1)$$

were I_o and I are the fluorescence intensities in the absence and in the presence of total quencher concentration $(Q)_T$, τ_F is the unquenched fluorescence lifetime, k_q is the bimolecular quenching rate constant, and K_{SV} is the Stern-Volmer constant. For a quenching molecule which partitions into the micellar phase, the value of $(Q)_T$ may be taken, to a first approximation, as the concentration of Q in the micellar phase. The partition coefficient, K_P is defined as follow:

$$K_P=(Q)_M/(Q)_A \qquad (2)$$

where Q_A and Q_M refer to the quencher in the aqueous and micellar phase, respectively. Thus, the Stern-Volmer relationship becomes

$$I_o/I-1=K_{SV}K_P(Q)_A \qquad (3)$$

Since the total volume of the system (V_T) consists of the volume of aqueous (V_A) and micellar components (V_M), then it follows

$$V_T(Q)_T=V_A(Q)_A+V_M(Q)_M \qquad (4)$$

Substitution of eq.2 and eq.4 into eq.3 yields eq. 5:

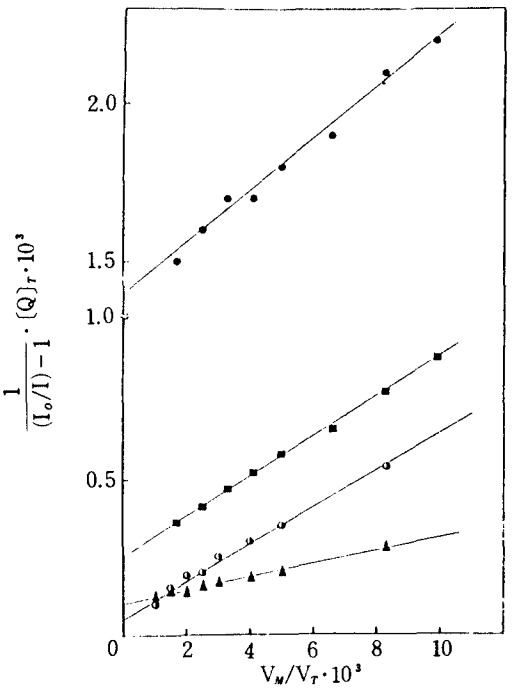


Fig4. Quenching of AS in Zephiramine micellar solution by Aniline(●), N-methylaniline (■), N,N-dimethylaniline(▲), and N, N-diethylaniline (◑) at room temperature plotted according to eq.(6).

$$I_o/I-1=\frac{K_{SV}K_P V_T}{V_A+V_M K_P}(Q)_T \quad (5)$$

For micellar systems $V_T \cong V_A$ and eq.3 may then be rearranged to give eq.6.

$$\frac{I}{I_o-I}(Q)_T=\frac{1}{K_{SV}}\frac{V_M}{V_T}+\frac{1}{K_{SV}K_P} \quad (6)$$

By plotting the left hand side of eq.6 against V_M/V_T, the value of K_{SV} can be determined from the slope and K_P from the ordinate intercept. The volume of the surfactant micelle was calculated from the known partial specific volume (17, 18) for use in eq.6.

It should be emphasized that K_P derived by this method is an "apparent" partition coefficient as it reflects the relative proximity of the fluorophore to the quencher rather than any absolute measure of quenchers partitioning between micellar and aqueous phases (19). Thus K_P reflects the local concentration of the quencher surrounding the fluorophore. Therefore the ratio of K_P of the solubilizate in the surface area (K_P^s) to K_P in the interior (K_P^{in}) of the surfactant micelle becomes same as the ratio of the concentration of the solubilizate in the surface area (C^s) to the concentration in the interior (C^{in}) of surfactant micelle, as following:

$$K_P^s/K_P^{in}=C^s/C^{in}$$

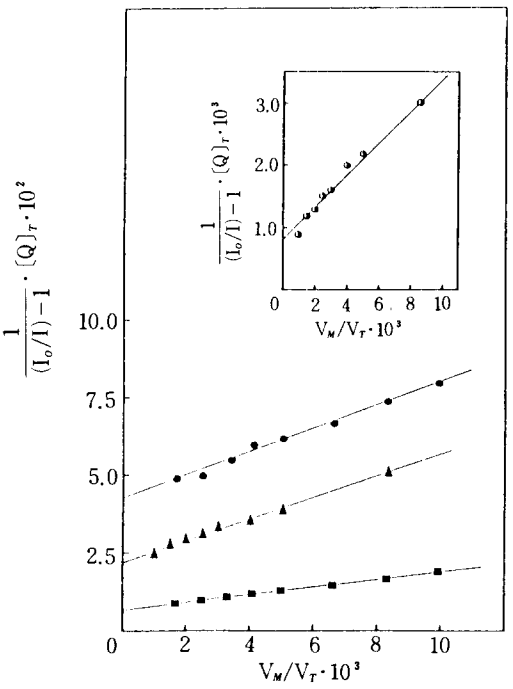


Fig.5. Quenching of TNS in Zephiramine micellar solution by Aniline(●), N-methylaniline(■), N,N-dimethylaniline(▲), and N,N-diethylaniline(◑) at room temperature plotted according to eq.6.

Table I. Parameters Associated with the Quenching of AS and TNS by Various Solubilizates in Aqueous Micellar Solutions of Triton X-100 and Zephiramine at Room Temperature.

Surfactant	Solubilizate	K_{SV}[a] AS	K_{SV}[a] TNS	K_P[b] AS	K_P[b] TNS
Triton X-100	Aniline	16.7	1.4	15.2	13.8
	N-methylaniline	40.5	1.0	131.4	36.4
	N, N-dimethylaniline	26.7	3.4	108.6	22.3
	N, N-diethylaniline	32.7	24.0	278.2	23.8
Zephiramine	Aniline	11.8	0.2	60.7	98.1
	N-methylaniline	50.0	0.3	181.8	163.6
	N, N-dimethylaniline	15.6	0.9	256.0	162.9
	N, N-diethylaniline	16.7	3.6	1200.0	350.0

[a] Stern-Volmer constant, M^{-1}. [b] Partition coefficient.

Fig.2 and 3 show quenching of AS and TNS probes by aniline and its derivatives in the aqueous micellar solution of Triton X-100 at room temperature, respectively. Fig.4 and 5 show the results in the aqueous micellar solution of Zephiramine. These figures were plotted according to eq. 6. K_P and K_{SV} values of the solubilizate were obtained from the intercept and the slope of these plots, and the results are listed in Table I. K_P values became larger gradually as the solubilizate has more numbers of N-substituents of aniline and more carbon numbers in the substituents in both cases of AS and TNS. This indicates that the capacity of solubilization into the micelle is dependent on the hydrophobicity of the solubilizate and that the solubilizate which are highly hydrophobic predominates in the ability to penetrate into the micelle. However, this also indicates that there is uneven distribution of the solubilizate at a graded series of levels in micelle.

Table III shows the ratio of the concentration of the solubilizate in the surface area to that in the interior of the micelle, C^{in}/C^s which were obtained on the basis of eq.7. These values ranged from 0.6 to 11.7 and were not extremely small or infinitely large. This behavior can be ra onalized as that there is not a definite position for tne solubilization of aniline and its derivatives in the micelle, and on the contrary, they are distributed in the multiple -sites of the micelle. C^{in}/C^s values became larger

Table II. The Ratios of the Concentration of Solubilizate in the Surface Area to That in the Interior of Surfactant Micelle.

Solubilizate	C^{in}/C^{s}	
	Triton X-100	Zephiramine
Aniline	1.1	0.6
N-methylaniline	3.6	1.1
N, N-dimethylaniline	4.9	1.6
N, N-diethylaniline	11.7	3.4

gradually in proportion to the numbers of N-substituent groups of aniline and carbons in the substituent. This indicates that the solubilization site of the solubilizate is also dependent on its structure. From the results of this study, it can be concluded that as the solubilizate has more numbers of N-substituents of aniline and the more numbers of carbons in the substituents, it tends to incorporate in the interior of the micelle more effectively. This might be due to the higher hydrophobicity of the solubilizate and thus to the more powerful hydrophobic interaction between the solubilizate and the hydrophobic interior of the micelle.

C^{in}/C^{s} values in case of Triton X—100 were larger than those of Zephiramine for all solubilizates. Recently, the size and shape of the Triton X-100 micelle have been of considerable controversy. Robson and Dennis (20) and Paradies (21) have postulated an oblate ellipsoid model for the Triton X-100 micelle. According to this model, the hydrophobic groups and the hydrophilic groups of Triton X-100 can separate from each other and each layer packs well. Consequently, this model predicts a more hydrophobic and more viscous core for the Triton X-100 micelle because of its well-packed structure. All of these studies indicate that Triton X-100 forms considerably large micelles (MW=63000-150000) compared with ionic micelles (MW=18000 for sodium dodecyl sulfate (SDS) and 26000 for hexadecyltrimethylammonium chloride (HTAC)). The interior of the Triton X-100 micelle where the probe molecules are located is less fluid and less polar than those of the SDS and HTAC micelles. The higher ability of the interior of the Triton X-100 micelle to solubilize these hydrophobic solubilizates may be ascribed to these natures of the Triton X-100 micelle.

LITERATURE CITED

1. Mittal, K.L. and Fendler. E.J. : "Solution Behavior of Surfactants", Vol.1, Plenum Press, New York, p.15(1982).
2. Matsuo, T., Yudate, K. and Nagamura, T. : NMR and paramagnetic-and fluorescent-probe studies on solubilization site in cationic surfactant micelles containing phenoxy groups. *J. Colloid Interface Sci.* **83**, 354 (1981).
3. Eriksson, J.C. and Gillberg, G. : NMR-studies of the solubilisation of aromatic compounds in cetyltrimethylammonium bromide solution II. *Acta Chem. Scand.* **20**, 2019(1966).
4. Fendler, J.H., Fendler, E.J., Infante, G.A., Shih, P. and Patterson, L.R. : Absorption and photon magnetic resonance spectroscopic investigation of the environment of acetophenone and benzophenone in aqueous micellar solutions. *J. Am. Chem. Soc.* **87**, 89(1975).
5. Jobe, D.J., Reinshorough, V.C. and White, P. J. : Solubilization sites in micellar sodium octylsulphate solutions by ultrasonic spectroscopy. *Can. J. Chem.* **60**, 279(1982).
6. Elworthy, P.H., Florence, A.T. and Macfarlene, C.B. : "Solubilization by surface-active agents and its applications in Chemistry and Biological Sciences", Champman and Hall, London, p.61(1968).
7. Cardinal, J.R. and Mukerjee, P. : Sol. nt effects on the ultraviolet spectra of benzene derivatives and naphthalene. Identification of polarity sensitive spectral characteristics. *J. Phys. Chem.* **82**, 1614(1978).
8. Mukerjee, P. and Cardinal, J.R. : Benzene derivatives and naphthalene solubilized in micelles. Polarity of microenvironment, location and distribution in micelles, and correlation with surface activity in hydrocarbon-water systems. *J. Phys. Chem.* **82**, 1620(1978).
9. Pownall, H.J. and Smith, L.C. : Viscosity of the hydrocarbon region of micelles. Measurement by excimer fluorescence. *J. Am. Chem. Soc.* 95, 3136(1973).
10. Kalyanasundaram, K. and Thomas, J.K. : Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. *J. Am. Chem. Soc.* **99**, 2039(1977).
11. Turro, N.J. and Yekta, A. : Luminescent probes for detergent solutions. A simple procedure for determination of the mean aggregation number of micelles. *J. Am. Chem. Soc.* **100**, 5951(1978).
12. Almgren, M. Grieser, F. and Thomas, J.K. : Dynamic and static aspects of solubilization of neutral arenes in ionic micellar solutions. *J. Am. Chem. Soc.* **101**, 279(1979).

13. Chalpin, D.B. and Kleinfeld, A.M. : Interaction of fluorescence quenchers with the n-(9-anthroyloxy) fatty acid membrane probes. *Biochim. Biophys. Acta.* **731**, 465(1983).
14. Azzi, A. : The application of fluorescent probes in membrane studies. *Quarterly Reviews of Biophysics* **8**, 237(1975).
15. Koblin, D.D., Pace, W.D. and Wang, H.H. : The penetration of local anesthetics into the red blood cell membrane as studid by fluorescence quenching. *Arch. Biochem. Biophys.* **171**, 176(1975).
16. Sikaris, K.A., Thulborn, K.R. and Sawyer, W.H. : Resolution of partition coefficients in the transverse plane of the lipid bilayer. *Chem. Phys. Lipids* **29**, 23(1981).
17. Jain, M.K. and Wagner, R.C. : "Introduction to Biological Membranes", Wiley, New York, p.67(1980).
18. Ekwall, P., Mandell, L. and Solyom, P. : The aqueous cetyltrimethylammonium bromide solutions. *J. Colloid Interface Sci.* **35**, 579(1971).
19. Blatt, E., Ghiggino, K.P. and Sawyer, W. H. : Fluorescence depolarization studies of n-(9-anthroyloxy) fatty acids in cetyltrimethylammonium bromide micelles. *J. Phys. Chem.* **86**, 4461(1982).
20. Robson, R.J. and Dennis, E.A. : The size, shape, and hydration of nonionic surfactant micelles. Triton X-100. *J. Phys. Chem.* **81**, 1075(1977).
21. Paradies, H.H. : Shape and size of a nonionic surfactant micelle. Triton X-100 in aqueous solution. *J. Phys. Chem.* **84**, 599(1980).