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Micellar Interaction of Tetracycline Antibiotics[1)]

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Micellar interactions of tetracycline, oxytetracycline, chlortetracycline and minocycline were examined in aqueous solutions of polyoxyethylene lauryl ether (PLE), sodium lauryl sulfate (SLS) and dodecyltrimethylammonium chloride (DTAC) at various pHs (2.1—5.6) by dynamic dialysis. The respective partition coefficients of ionized and zwitterionic species were calculated. The ionized species of the antibiotics except for minocycline were more solubilized than zwitterionic ones in PLE solution, in which the orientation of hydrophobic parts of the antibiotic and micelle structures plays a primary mechanism for their interaction. In ionic surfactant solutions, the electrostatic force was predominant at lower pH and the solubilization capability of the anionic surfactant was much higher than that of the cationic one for tetracycline over the pH range examined.

Keywords——solubilization by surfactant; solubilization of ionized form; hydrophobic interaction; dynamic dialysis; tetracyclines; polyoxyethylene lauryl ether; sodium lauryl sulfate; dodecyltrimethylammonium chloride

In pharmaceutical preparations, various surfactants have been used to formulate dispersed and solubilizing systems. In particular, nonionic surfactants have often been employed for such preparations because they are less toxic to biological systems.[3)] However, it is known that drug-surfactant interactions modify the rate of intestinal absorption of drugs,[4)] which has brought the necessity for further knowledges and accumulations of case involving such interaction.

Naggar and coworkers[5)] investigated the solubilization of tetracycline and oxytetracycline by polysorbate 20 and 80 and polyethylene glycol at pH 5.0. They assumed that these interactions were due to some type of complexation, and the method of Higuchi and Lach,[6)] assuming 1:1 complex formation, was simply applied to the determination of the stability constant.

Tetracycline derivatives exist in solution as zwitter, positively charged and/or negatively charged species as a function of pH.[7)] The respective species, therefore, might yield to some extent different micellar binding in surfactant solution. The objective of this study is to describe the effect of pH on the binding of tetracycline derivatives to micelles of various surfactants and to extend discussions to a possible mechanism involved.

Experimental

Materials——Tetracycline hydrochloride, chlortetracycline hydrochloride and minocycline hydrochloride were donated by Japan Lederle Ltd. Oxytetracycline hydrochloride was the gift of Taito Pfizer Ltd. These antibiotics were used without further purification. Polyoxyethylene lauryl ether (PLE) was purified from commercially available Brij 35 by the method described previously[8)] and only a single spot was observed

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3) B.A. Mulley, "Advances in Pharmaceutical Sciences," Academic Press, London, 1964, p. 164.
4) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **59**, 579 (1970).
5) V. Naggar, N.A. Daabis, and M.M. Motawi, *Pharmazie*, **29**, H.2, 122 (1974).
6) T. Higuchi and J.L. Lach, *J. Am. Pharm. Ass., Sci. Ed.*, **43**, 349 (1954).
7) L.J. Leeson, J.E. Krueger, and R.A. Nash, *Tetrahedron Letters*, **1963**, 1155.
8) K. Ikeda, T. Kato, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2510 (1971).

by the Nakagawa and Nakata thin-layer chromatographic method.[9] Sodium lauryl sulfate (SLS) was recrystallized from *n*-butanol after extraction of higher alcohols with ether for 35 hours. Dodecyltrimethylammonium chloride (DTAC) was recrystallized from acetone. The membrane used for dynamic dialysis was a Visking cellulose tube boiled in distilled water for 30 minutes before use. Phosphate buffers were used for the preparation of all pH solutions. The total buffer concentration was kept at 0.05M for each preparation, and sodium chloride was added to adjust the ionic strength to be 0.1. All other materials and solvents were of analytical grade.

Assay of Tetracycline Derivatives——Tetracycline derivatives were assayed spectrophotometrically at their respective absorbance maxima which range from 352 to 367 nm. Under the present experimental conditions no ultraviolet (UV) spectral change was observed with time for all systems.

Determination of Partial Molar Volume of Micelles——A Lipkin-Davison type pycnometer was used for the determination of the density of aqueous surfactant solution, from which the partial molar volumes of PLE, SLS and DTAC in pH 2.1 and 5.6 phosphate buffer solutions were calculated (25°). They are

	PLE	SLS	DTAC
aq. dist.	1028 ml/mole	243	286
pH 2.1	1110	261	286
pH 5.6	1090	252	276

Dynamic Dialysis Method——Dynamic dialysis was carried out at 25±0.5° by the method employed previously.[8] A diffusion cell, consisting of two chambers separated by dialysis membrane, was used for the determination of the permeation rate of free drug. The diameter of the membrane was 4.2 cm. The total antibiotic concentration in the donor chamber was always kept at 2.0×10^{-3}M in all pH surfactant solutions. The surfactant concentrations were 3.3×10^{-2}M in the case of PLE, 1.0×10^{-2}M in SLS and 2.5×10^{-2}M in DTAC, respectively. The permeation rate is proportional to the concentration of free drug on the assumption that the drug bound in micelles is impermeable. When the concentration of the permeating drug increases linearly with time, the following relationship can be obtained:

$$\frac{\text{Permeation rate in the presence of surfactant}}{\text{Permeation rate in the absence of surfactant}} = \frac{1}{1-v}\cdot\frac{D_w}{D_w+D_m} \qquad (1)$$

where v is the volume fraction of the micellar phase. D_w and D_m are the amount of free drug in the surfactant solution and that of the drug bound in the micelle, respectively.

By applying the Langmuir type plot to the interaction of a drug with surfactant the following equation is obtained:

$$r = \frac{K_1K_2C_f}{1+K_2C_f} \qquad (2)$$

where r is the number of drug molecules bound to one surfactant molecule. K_1 and K_2 are the constants and C_f is the concentration of free drug.

Results and Discussion

Various methods, *e.g.* equilibrium dialysis,[10] dynamic dialysis,[8] potentiometric titration[11] and molecular sieve[12] have been available for the quantitative study of micellar interaction of a drug. Among them dynamic dialysis method was found to be the most reliable for the tetracycline antibiotics.

To apply the partition law to the relation between the amount of drug and the surfactant concentration, the Langmuir plot was made for all antibiotic-surfactant systems. Fig. 1 represents a typical plot for the sloubilization of tetracycline in PLE solution, in which linearity was established for the range of the drug concentration examined. For all other antibiotic-surfactant cases, similar linearity was obtained at all pH values.

The linearity indicated that the partition theory was applicable for the solubilization so that the apparent partition coefficient, K_{app}, was defined by

9) T. Nakagawa and I. Nakata, *Kogyo Kagaku Zasshi*, **59**, 710 (1956).
10) N.K. Patel and N.E. Foss, *J. Pharm. Sci.*, **54**, 1495 (1965).
11) M. Donbrow and C.T. Rhodes, *J. Chem. Soc.*, **1964**, 6166.
12) M. Donbrow, E. Azaz, and R. Hamburger, *J. Pharm. Sci.*, **59**, 1427 (1970).

$$K_{app} = \frac{D_m/v}{D_w/(1-v)} \quad (3)$$

Table I shows the pH dependency of K_{app} in PLE solution estimated from equations 1 and 3. K_{app} for tetracycline, oxytetracycline and chlortetracycline became smaller as pH increased while minocycline showed in an opposite manner.

Tetracycline derivatives have three macroscopic dissociation constants.[13] The first dissociation constant is related with the tricarbonylmethane moiety, the second with the phenolic diketone group and the third with dimethylamino moiety, as shown in Fig. 2. Among the tetracycline derivatives only minocycline carries an additional dimethylamino group at carbon 7 as the fourth dissociation function. In the pH range studied (2.1—5.6), the species of tetracycline derivatives converts from the cationic form, (I°, II°, III^{+}), to the zwitterionic, (I^{-}, II°, III^{+}), which represent the most protonated species and the one with the loss of a proton from the tricarbonylmethane system, respectively.

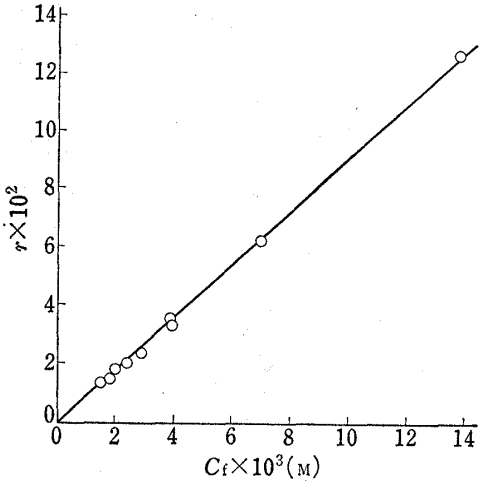

Fig. 1. Langmuir Plot for Tetracycline Interaction with PLE obtained by Dynamic Dialysis Method at pH 3.0 and 25°

Table I. Apparent Partition Coefficients, K_{app}, in PLE Solution at Various pH's(25°)

Substance	pH 2.1	3.0	3.9	5.6
Tetracycline	8.05	8.64	6.31	5.80
Oxytetracycline	8.01	7.61	6.54	5.68
Chlortetracycline	19.0	17.9	13.3	10.0
Minocycline	2.1	4.1	3.8	17.0

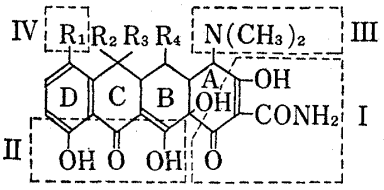

Fig. 2. Three or Four Functional Groups Associated with Macroscopic Dissociation Constants of Tetracycline Derivatives

tetracycline:
R_1=H, R_2=CH_3, R_3=OH, R_4=H
oxytetracycline:
R_1=H, R_2=CH_3, R_3=OH, R_4=OH
chlortetracycline:
R_1=Cl, R_2=CH_3, R_3=OH, R_4=H
minocycline:
R_1=$N(CH_3)_2$, R_2=H, R_3=H, R_4=H

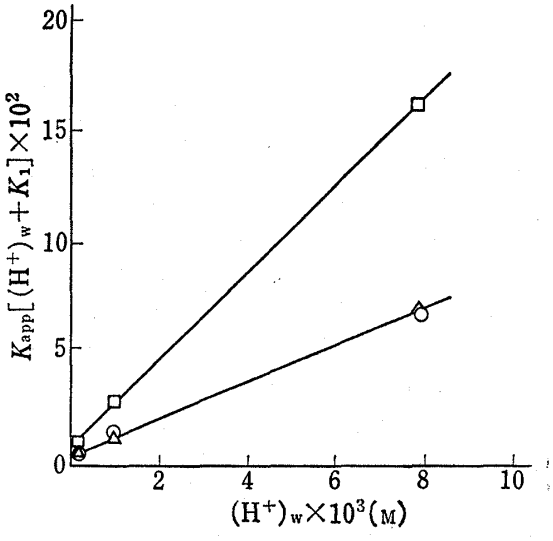

Fig. 3. Relationship between $K_{app}[(H^+)_w+K_1]$ and $(H^+)_w$ in PLE Solution

○: tetracycline, △: oxytetracycline, □: chlortetracycline

13) W.C. Barringer, W. Shultz, M. Sieger, and R.A. Nash, *Am. J. Pharm.*, **146**, 179 (1974).

When both cationic and zwitterionic forms contribute to the magnitude of K_{app}, the state of equilibrium may be represented as follows:

$$(\bigcirc, \bigcirc, +)_m \overset{K_c}{\rightleftarrows} (\bigcirc, \bigcirc, +)_w \overset{K_1}{\rightleftarrows} (-, \bigcirc, +)_w \overset{K_z}{\rightleftarrows} (-, \bigcirc, +)_m$$

where K_1 is the dissociation constant of tetracycline derivatives and K_c and K_z are the partition coefficients of the protonated and the zwitterionic species, respectively. The subscripts w and m denote the aqueous and micellar phases, respectively. These constants may be expressed in terms of the concentrations of respective species and the volume fractions:

$$K_c = \frac{(\bigcirc, \bigcirc, +)_m}{(\bigcirc, \bigcirc, +)_w} \cdot \frac{1-v}{v} \tag{4}$$

$$K_z = \frac{(-, \bigcirc, +)_m}{(-, \bigcirc, +)_w} \cdot \frac{1-v}{v} \tag{5}$$

$$K_1 = \frac{(-, \bigcirc, +)_w (H^+)_w}{(\bigcirc, \bigcirc, +)_w} \tag{6}$$

On the other hand, the apparent partition coefficient may be generally given by

$$K_{app} = \frac{(\bigcirc, \bigcirc, +)_m + (-, \bigcirc, +)_m}{(\bigcirc, \bigcirc, +)_w + (-, \bigcirc, +)_w} \cdot \frac{1-v}{v} \tag{7}$$

Substituting equations 4, 5 and 6 into 7 yields

$$K_{app}[(H^+)_w + K_1] = K_c(H^+)_w + K_1 K_z \tag{8}$$

Equation 8 was evaluated by using the data for the tetracyclines except for minocycline, as shown in Fig. 3. K_c and K_z, therefore, were estimated from the slope and the intercept on the ordinate.

Table II. Dissociation Constants, K_1 and Partition Coefficients, K_c and K_z at 25°

	$K_1 \times 10^4$	K_c	K_z
Tetracycline	4.68	8.15	6.76
Oxytetracycline	5.37	8.14	6.18
Chlortetracycline	5.37	19.4	12.3

Table II shows that K_C was appreciably bigger than K_z, indicating that the cationic form was more solubilized than the zwitterionic one for the antibiotics listed. Meanwhile, it has been indicated that a number of ionized drugs were not solubilized by nonionic surfactants,[11,14,15] but Biber and Rhodes suggested that there might be a micellar bound case if an ionic form carried a relatively large hydrophobic portion in the molecule.[16] This finding may support their suggestion.

Colaizzi and Klink investigated partitioning behaviors of these antibiotics in *n*-octanol–water system as a function of pH,[17] describing that the zwitterionic species was more lipophilic than the cationic due probably to an effective cancellation of charge within the molecule. Assuming that the interior of the PLE micelle, to which the partition occurs, has a hydrophobic nature similar to the oil phase, the present results of K_{app} and subsequently calculated K_c and K_z for tetracycline, oxytetracycline and chlortetracycline are obviously contrary to the anticipation from their results. This indicates that the micellar

14) D.L. Dyer, *J. Colloid Sci.*, **14**, 640 (1959).
15) J.H. Collet and R. Withington, *J. Pharm. Pharmac.*, **24**, 211 (1972).
16) M.Z. Biber and C.T. Rhodes, *Acta Pharm. Suecica.*, **11**, 275 (1974).
17) J.L. Colaizzi and P.R. Klink, *J. Pharm. Sci.*, **58**, 1184 (1969).

interaction of the antibiotics could not be necessarily consistent with normal partitioning behavior due to lipophilicity of the species but naturally other interaction mechanisms such as hydrogen bonding and an incorporation into the structure of micelle as a mixed micelle component could arise. Polyoxyethylene surfactants have been known to interact with various acidic substances,[18,19] whose cases are attributed to hydrogen bonding. Therefore, it may be conceivable that an acidic proton belonging to the moiety I of the cationic form was involved in hydrogen bonding towards the oxygen atoms of polyoxyethylene molecule while the zwitterionic one has liberated a hydrogen ion from the moiety I, resulting in $K_c > K_z$.

On the other hand, many drugs, which are not primarily surfactant, have been found to aggregate to form micelles in aqueous solutions.[20] This implies the existence of a hydrophobic region in the molecule, which will be able to form hydrophobic bonds with surfactants and other drug molecules. Rozhanskaya, *et al.* showed that an association occurs in oxytetracycline solution at pH 1.65 when the concentration is more than 5×10^{-3} M.[21] Thus, another possible explanation is that the interaction is due to a mutual solution of the nonpolar parts of the species and the hydrocarbon parts of the micelle, occurring as a consequence of orientation of the species by their contact with aqueous environment and the hydrophobic nature of the micelle, and contributing to the formation of a mixed micelle. So it is very likely that the cationic forms of tetracycline, oxytetracycline and chlortetracycline were more solubilized by the orientation mechanism than the zwitterionic ones by the intramolecular cancellation of charges.

In the case of minocycline, although K_c and K_z were not calculated, K_{app} dependency on pH showed an entirely opposite trend compared with others, but similar tendency to the result of the *n*-octanol–water partitioning, in which no minocycline partitioned at pH 2.1.[17] Under this condition minocycline exists principally as a doubly protonated, and with increasing pH the antibiotic converts to the neutral zwitterionic form through the one having doubly protonated and negatively charged units. The fact is that the doubly protonated species of this antibiotic was able to interact with PLE micelle while it was unable to partition into the oil. Furthermore, it should be noted that the doubly protonated species of minocycline interacted to a lesser extent with PLE micelle than the single-protonated of the others by a factor of 4—10. This could be explained by what the doubly protonated species has more difficulty due to the position of the charged units, the dimethylamino groups in the moieties III and IV, for the orientation mechanism. The difference of K_{app} mentioned above may also suggest that the single-protonated species of tetracycline, oxytetracycline and chlortetracycline take predominantly the orientation mechanism rather than hydrogen bonding beacuse the moiety I of minocycline also bears the same kind acidic proton as the three others at pH 2.1.

The interaction behavior of tetracycline with SLS and DTAC micelles was examined in the same manner at various pH's. As shown in Table III, the dependencies of respective

Table III. Apparent Partition Coefficients, K_{app}, of Tetracycline in SLS and DTAC Solutions at Various pH's (25°)

Solution	pH 2.1	3.0	3.9	5.4
Sodium lauryl sulfate	2860	2690	1130	390
Dodecyltrimethylammonium chloride	0	13	15	18

18) T. Higuchi and J.H. Lach, *J. Am. Pharm. Ass., Sci Ed.*, **43**, 465 (1954).
19) D. Guttman and T. Higuchi, *J. Am. Pharm. Ass., Sci Ed.*, **45**, 659 (1956).
20) A.T. Florence, *Advan. Colloid Interface Sci.*, **2**, 115 (1968).
21) T.I. Rozhanskaya, L.V. Dmitreko, G.B. Selekhova, and G.V. Samsonov, *Kolloid. Zh.*, **36**, 58 (1974).

K_{app} on pH exhibited a marked contrast: in the case of SLS solution K_{app} decreased as pH increased and the DTAC case appeared in an opposite trend. At pH 2.1, a considerably high degree of interaction was observed in the anionic surfactant solution. This is expected because the attraction due to the electrostatic force between the protonated species and the anionic surfactant micelle became great enough, and then followed by the decrease of K_{app}, that the decrease of fraction of the protonated species, as pH increases. Unlike the anionic case, no interaction occurred for the DTAC micelle at pH 2.1, which indicates the electrical repulsive force exclusively plays a significant role between the protonated species and the cationic surfactant micelle presenting a positively charged surface to the aqueous environment.

The interaction of tetracycline with PLE, SLS and DTAC micelles was examined by the UV spectral method at pH 2.1 and 5.6. The λ max at 355—356 nm assigned for the rings B, C and D showed a red shift in the surfactant solutions while the λ max at 270 nm assigned for the rings A, B, C and D remained unchanged,[22] as shown in Table IV. A

Table IV. Ultraviolet Absorption Characteristics of Tetracycline in Various Solvents

Solvents	λ_{max} nm	λ_{max} nm
Water (2.1)[a]	355	268
(5.6)	356	275
PLE (2.1)	358	268
(5.6)	358	275
SLS (2.1)	362	268
(5.6)	360	275
DTAC (2.1)	356	268
(5.6)	358	275
Cyclohexane	365	263
n-Octanol	366	263

a) Numeric in parentheses shows pH.
PLE: 3.3×10^{-2}M, SLS: 1.0×10^{-2}M, DTAC: 2.5×10^{-2}M

similar red shift concerning 355 nm max was observed in cyclohexane and *n*-octanol solutions. This suggests that the most part of the molecule is located in the hydrophobic environment and the ring A bearing a positively charged moiety at pH 2.1 and both positively and negatively charged moieties at pH 5.6, respectively, are oriented to the hydrophilic outer part of the micelle.

In conclusion, this study has demonstrated that the ionized form of a drug was more solubilized by nonionic surfactant than its electrically neutral form, which could be explained by what in the case of tetracycline, oxytetracycline and chlortetracycline–PLE micelle systems their ionic form carried a large hydrophobic portion in the molecule and was more easily incorporated in the PLE micelle, resulting in a mixed micelle formation. In this respect, pharmaceutical formulation studies must take into account the type of phenomena shown in this paper when dealing with a drug having hydrophobic part in the molecule and a complex dissociation function. Furthermore, it should be noted that pH dependency of these tetracycline–PLE micelle interactions did not correlate with the result of the *n*-octanol–water partitioning, although the *n*-octanol–water partition coefficients have been useful in predicting the membrane-water partition and the pH-partition behavior in drug absorption studies.

22) J.R.D. McCormick, S.M. Fox, L.L. Smith, B.A. Bither, J. Reichenthal, V.E. Origori, W.H. Muller, R. Winterbottom, and A.P. Doerschuk, *J. Am. Chem. Soc.*, **79**, 2849 (1957).