Chem. Pharm. Bull. 28(6)1768—1774(1980)

The Interactions of Prostaglandins E_1, E_2 and $F_{2\alpha}$ with Lauromacrogol[1]

Seishi Oguri, Toshihisa Yotsuyanagi, and Ken Ikeda

Faculty of Pharmaceutical Sciences, Nagoya City University[2]

(Received December 17, 1979)

The interaction of unionized and ionized species of prostaglandins (PGE_1, PGE_2 and $PGF_{2\alpha}$) with lauromacrogols was studied by equilibrium dialysis and potentiometric titration. The magnitude of interaction was analyzed in terms of the partition coefficient (P), moles of drug bound per mole of surfactant (K) and moles of drug bound per micelle (K'). The order of the interaction constants with lauromacrogol containing 23 mol of ethylene oxide was $PGE_1 > PGE_2 > PGF_{2\alpha}$, which corresponds to the order of their partition coefficients between water and cyclohexane. The effect of oxyethylene chain length on the interaction was examined with PGE_1 and $PGF_{2\alpha}$. Regardless of species, the K value increased with increasing oxyethylene chain length. The P and K' values decreased with increasingly hydrophilic micellar phase. This is due to an increase of the partial molar volume and a reduced aggregation number, leading to an increase of micellar concentration with increasing oxyethylene chain length. The measures of the degree of interaction are desired to be closely related to the changes of physicochemical characteristics of micelles, *i.e.*, to the amounts of drugs in the hydrophobic and hydrophilic regions available for drug binding.

Keywords——prostaglandins; lauromacrogol; micellar interaction; effect of oxyethylene chain length; partition coefficient of prostaglandins; equilibrium dialysis; potentiometric titration

Due to the complexities of their physicochemical properties, such as micelle formation, solubility behavior and stability in water,[3–5] prostaglandins require particular care and attention to detail in the development of rational formulations. Prostaglandins are categorized as very slightly soluble drugs, and their solubilities are highly dependent on the pH of an aqueous medium in relation to the pK_a values. Therefore, the solubilization of prostaglandins appears to be a worthwhile object of study.

The present study was undertaken to investigate the interactions of prostaglandins E_1, E_2 and $F_{2\alpha}$ with lauromacrogols in terms of the pH dependent chemical species. Furthermore, the relationship between the magnitude of the interaction and the ethylene oxide chain length of the surfactants was investigated based on the stoichiometric treatment and the partition model.

Experimental

Materials——Prostaglandins E_1 (PGE_1), E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) were gifts from Ono Pharmaceutical Co. Ltd., and were used without further purification. Lauromacrogols were purified from commercially available Brij 35, and Actinol[6] L10, L15, L20, L25 and L50 by the method described previously.[7] Buffer substances and all other chemicals were of reagent grade. Visking cellulose tubing (27/32) used for dialysis was washed in boiled distilled water before use.

1) Presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1979.
2) Location: *3-1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.*
3) T.J. Roseman and S.H. Yalkowsky, *J. Pharm. Sci.*, **62**, 1680 (1973).
4) M.C.R. Johnson and L. Saunders, *Biochim. Biophys. Acta*, **218**, 543 (1970).
5) K. Uekama and F. Hirayama, *Chem. Pharm. Bull.*, **26**, 1195 (1978).
6) Polyoxyethylene lauryl ethers kindly supplied by Matsumoto Oil and Wax Co. Ltd., Osaka, Japan.
7) K. Ikeda, T. Kato, and T. Tsukamoto, *Chem. Pharm. Bull.*, **19**, 2510 (1971).

NII-Electronic Library Service

Determination of the Average Number of Ethylene Oxide Units by ^{1}H-NMR——The average number of ethylene oxide units (n) was determined from the ^{1}H-NMR spectra in aqueous solution. The value of n is presented below for each surfactant, which is abbreviated as PLEn:

	Actinol				Brij
	L10	L15	L20	L25	L50	35
Abbre.	PLE11	PLE18	PLE28	PLE32	PLE63	PLE23
n	11	18	28	32	63	23
N	88	52	32	29	11	39

The aggregation number (N) listed was calculated by means of Becher's equation, which describes a relationship between the aggregation number and the oxyethylene chain length.[8]

Assay of PGE and PGF$_{2\alpha}$——PGE$_1$ and PGE$_2$ were allowed to stand in 0.2 N NaOH for 90 min at 25° and were ultimately converted to the corresponding B type series.[9] They were then assayed spectrophotometrically at 283 nm. PGF$_{2\alpha}$ was studied by potentiometric titration.

Equilibrium Dialysis Method——An aliquot (10 ml) of PLE n solution (1×10^{-2}M) was enclosed in a dialysis bag and allowed to equilibrate with external buffer solution (40 ml) in a 50 ml stoppered test tube. PGE was dissolved in either the internal phase or external phase since the solubility of PGE in the low pH range is extremely low, whereas a relatively high concentration can be obtained in PLE solution. Total PGE concentrations ranged from 1×10^{-6} M at low pH case to 1×10^{-3} M at higher pH. The tubes were gently shaken in the dark in a thermostated water bath at 25° for 24 hours. Buffer solution used were 0.2 M KCl–HCl for pH 1.0—2.5, 0.1 M CH_3COONa–HCl for pH 2.8—5.1 and 0.3 M NaH_2PO_4–Na_2HPO_4 for pH 5.3—8.0.

Potentiometric Titration——PGF$_{2\alpha}$ (8×10^{-4} M) dissolved in PLEn solution of various concentrations (25 ml) was titrated with 2×10^{-2} N NaOH using a pH-stat apparatus (Toa Electronics Ltd., Model HS-2A). Titration was always performed under nitrogen at 25°. The initial ionic strength was adjusted to 0.2 by adding NaCl.

Determination of the Partial Molar Volume of Lauromacrogol Micelles——A Lipkin-Davison type pycnometer was used to determine the density of the surfactant, and the partial molar volumes (PMV) of PLEn at 25° then calculated to be as follows:

	PMV (ml/mol)		PMV (ml/mol)
PLE11	640	PLE28	1280
PLE18	900	PLE32	1430
PLE23	1100	PLE63	2580

It is known that the partial molar volume of PLEn is little affected by the pH and ionic strength of the medium.[10]

Quantitative Treatment of Interactions——(1) When a drug is considered to be adsorbed onto a micellar surface, the Langmuir equation may be utilized to describe the interaction:

$$r = \frac{k_1 k_2 C_f}{1+k_1 C_f} \tag{1}$$

where r is the number of moles of bound drug molecule per mole of surfactant. k_1 and k_2 are constants and C_f is the concentration of free drug.

(2) If the patition law is applicable between free and bound drugs, the partition coefficients (P) between the aqueous and micellar phases can be defined as

$$P = \frac{C_m}{C_a} = \frac{D_m/v}{D_a/(1-v)} \tag{2}$$

where C_a and C_m are the concentrations in the aqueous and micellar phases. D_a and D_m are the amounts in respective phases, and v is the volume fraction of the micellar phase.

(3) Assuming that drug and surfactant molecules form a complex stoichiometrically, the binding constant (K) is given by

$$K = \frac{C_b}{C_f(S-\mathrm{CMC})} \tag{3}$$

By introducing the aggregation number, it can be expressed in terms of the micellar and drug concentrations:

$$K' = \frac{C_b}{C_.(S-\mathrm{CMC})/N} \tag{4}$$

8) P. Becher, *J. Colloid Sci.*, **16**, 49 (1961).
9) N.H. Anderson, *J. Lipid Res.*, **10**, 320 (1969).
11) K. Ikeda, H. Tomida, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, **25**, 1067 (1977).

NII-Electronic Library Service

where C_b and S are the concentrations of bound drug and of the surfactant, respectively. CMC is the critical micelle concentration. Accordingly, $K'=K\cdot N$. Introducing the partial molar volume of the surfactant into eqs. (2) and (3), one can obtain the following relationship between K and P:

$$P = \frac{K}{\text{PMV}} \tag{5}$$

Results and Discussion

Interactions of PGE_1, PGE_2 and $PGF_{2\alpha}$ with PLE23

Fig. 1 shows typical plots of the r value against C_f based on eq. (1) under various pH conditions. For all pHs of the surfactant solutions studied, straight lines passing through the origin were obtained over the range of PGE_1 concentration examined. This indicates that K and P in eqs. (2) and (3) can be taken as measures of the magnitude of the interaction that are independent of the drug concentration. For other surfactants with different oxyethylene chain lengths, the r values were linearly related to the concentration of the free form at various pHs.

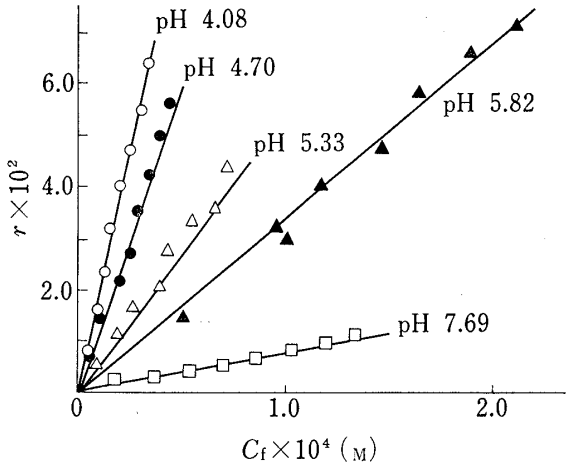

Fig. 1. Langmuir Plot for PGE_1 Interaction with PLE23, obtained by the Equilibrium Dialysis Method at 25°

PLE Concn.: 1×10^{-2} M.

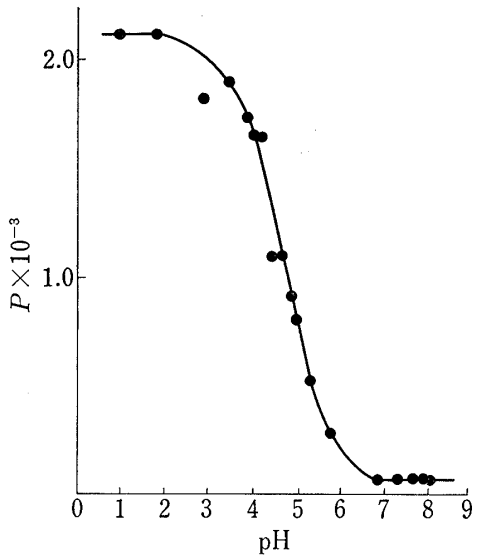

Fig. 2. The pH Dependency of the Partition Coefficient of PGE_1 in the PLE23 Micellar Phase at 25°

Ionic strength: 0.2.

The pH dependency of the P value was examined using the combination of PGE_1 and PLE23, as shown in Fig. 2. A reverse sigmoid curve, which is common for the water-oil partitioning of weakly acidic drugs, was observed.[11] As could be predicted from the pK_a value of PGE_1 ($pK_a=5.02$ at 25°), the unionized species predominates at pH<2, where the curve is horizontal. The partition coefficient of the unionized PGE_1 was calculated to be 2140. As the pH was increased ,the curve showed a sharp decrease of the partition coefficient and became flat again at a point where the P value of the ionized species was estimated to be 76. The partition coefficients were not affected by the ionic strength in the range from 0.1 to 0.5. The P values of unionized and ionized species of PGE_2 and $PGF_{2\alpha}$ were also determined and are listed in Table I.

11) A.N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," Lea and Febiger, Philadelphia, 1969, p. 316.

NII-Electronic Library Service

Table I. Partition Coefficients and Binding Constants of Unionized and Ionized Species of Prostaglandins E_1, E_2 and $F_{2\alpha}$ with PLE23 at 25°

	P_u	P_i	K_u	K_i	K_u'	K_i'
PGE_1	2140	76	2353	83	92000	3200
PGE_2	1755	47	1929	51	75000	2000
$PGF_{2\alpha}$	1056	20	1160	20	45200	780

Surfactant: PLE23, aggregation number: 39, CMC: 6—9×10^{-6} M.

The order of the P values was found to be $PGE_1 > PGE_2 > PGF_{2\alpha}$ for correspondimg species. It appears that among these prostaglandins the introduction of hydrophilic groups such as a hydroxy group on the ring and a double bond in the hydrocarbon chain reduces the magnitude of the partition coefficient. In comparison with the intrinsic partition coefficients of these drugs determined in the water–cyclohexane systems,[12] it is of interest that the P values of the unionized species in the PLE23 micelles were about 400 to 500 times larger, and even the ionized species showed much grater affinities for the micellar phase. Ionized species, in general, hardly partition into oil and micellar phases, as seen in the case of ionized salicylic acid partitioning into micelles of polysorbate and Myrjs.[13] Unlike smaller molecules, however, prostaglandins carry bulky hydrophobic moieties in their structure, so that the hydrophobic interaction with surfactant molecules appears to be significant. Due to such interactions, the partition coefficients of prostaglandins are considered to be much larger than those of steroids[14a] and benzoic acid derivatives.[14b]

The K and K' values determined on the basis of a stoichiometric treatment are also listed in Table I. As the CMC of PLE23 is 6.0—9.1×10^{-5} M, it is negligible in the calculation of K values.

Potentiometric Titration of $PGF_{2\alpha}$

The presence of surfactant affected the titration curves of $PGF_{2\alpha}$ as a result of binding to the surfactant, as shown in Fig. 3.

The potentiometric method has been applied to the study of solubilization by surfactants.[15] The hydrogen ion concentration, $(H^+)_h$, at half-neutralization in a surfactant solution can be generally expressed by

$$(H^+)_h = \frac{K_a K_i}{K_u} + \frac{K_a(K_u - K_i)}{K_u[1 + K_u(PLEn)]} \tag{6}$$

where K_u and K_i are the binding constants of the unionized and ionized species, respectively. $(PLEn)$ is the concentration of the surfactant, and K_a is the dissociation constant of the drug.

When the drug is titrated at various concentrations of $PLEn$, mathematical manipulation leads to the following equation:

$$\frac{(PLEn)_1 - (PLEn)_2}{(H^+)_{h1} - (H^+)_{h2}} = \frac{K_u[1 + K_u(PLEn)]}{K_a(K_i - K_u)} \cdot (PLEn)_1 + \frac{1 + K_u(PLEn)_2}{K_a(K_i - K_u)} \tag{7}$$

where the subscripts 1 and 2 indicate two different concentrations of the surfactant. Assuming $(PLEn)_2 = 0$, eq. (7) can be simplified to

$$\frac{(PLEn)_1}{(H^+)_{h1} - (H^+)_{h2}} = \frac{K_u}{K_a(K_i - K_u)} \cdot (PLEn)_1 + \frac{1}{K_a(K_1 - K_u)} \tag{8}$$

12) K. Uekama, F. Hirayama, and H. Tanaka, *Chem. Pharm. Bull.*, **26**, 3779 (1978).
13) J.H. Collett and R. Withington, *J. Pharm. Pharmacol.*, **24**, 211 (1972).
14) *a*) H. Tomida, T. Yotsuyanagi, and K. Ikeda, *Chem. Pharm. Bull.*, **26**, 2832 (1978); *b*) H. Tomida, T. Yotsuyanagi, and K. Ikeda, *ibid.*, **26**, 2824 (1978).
15) M. Donbrow and C.T. Rhodes, *J. Pharm. Pharmacol.*, **15**, 233 (1963).

NII-Electronic Library Service

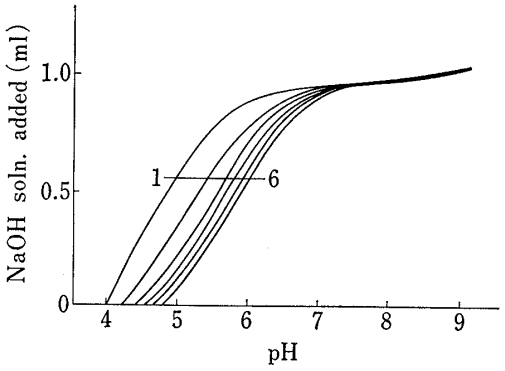

Fig. 3. Potentiometric Titration Curves of $PGF_{2\alpha}$ with NaOH (2×10^{-2} N) at 25°

$PGF_{2\alpha}$ Concn.: 8.00×10^{-4} M (25 ml).
$(PLE23)\times10^{3}$ (M)
1, 0
2, 2.0
3, 4,0
4, 6.0
5, 8.0
6, 10.0

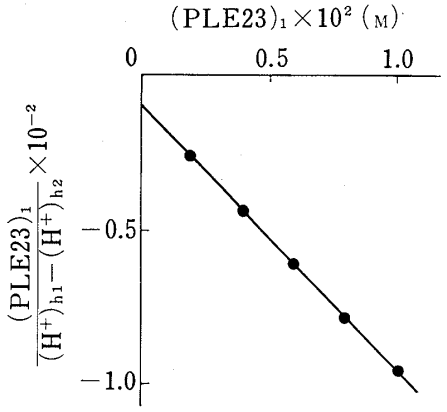

Fig. 4. Typical Plots of $(PLE23)_1/[(H^+)_{h1}-(H^+)_{h2}]$ against $(PLE23)_1$ for $PGF_{2\alpha}$ according to Equation (8)

A plot of $(PLEn)_1/[(H^+)_{h1}-(H^+)_{h2}]$ against $(PLEn)_1$ should give a straight line and K_u and K_i can readily be calulated from the slope and intercept values. Fig. 4 shows a typical plot for the titration record shown in Fig. 3.

For other surfactants with various oxyethylene chain lengths, the linear relationship based on eq. (8) is well established.

Effect of Oxyethylene Chain Length on the *P*, *K* and *K'* Values

The effect of oxyethylene chain length on the interactions of PGE_1 and $PGF_{2\alpha}$ was examined. As shown in Figs. 5, 6 and 7, P and K' decrease as the chain length increases, while the dependency of K was the reverse of this. Such trends were found whether the species was unionized or ionized.

The partial molar volume of the surfactant, as indicated in the experimental section, increases with oxyethylene chain length, while the aggregation number decreases.

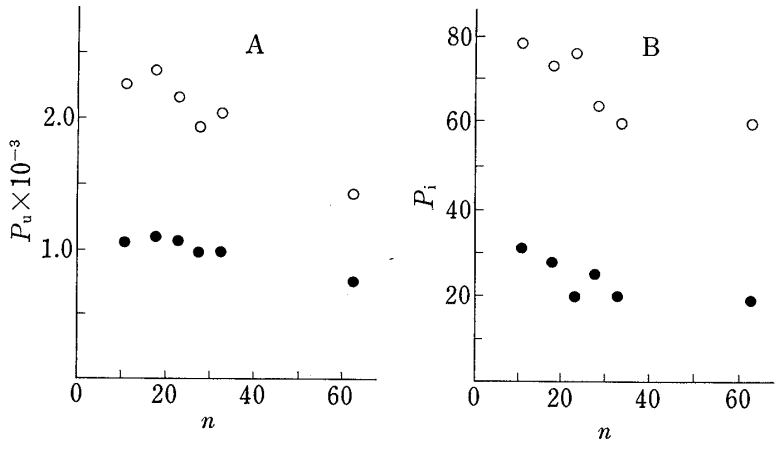

Fig. 5. Relationship between P and n at 25°

A) P_u at pH 2.04, B) P_i at pH 7.32.
○, PGE_1, ●, $PGF_{2\alpha}$.

NII-Electronic Library Service

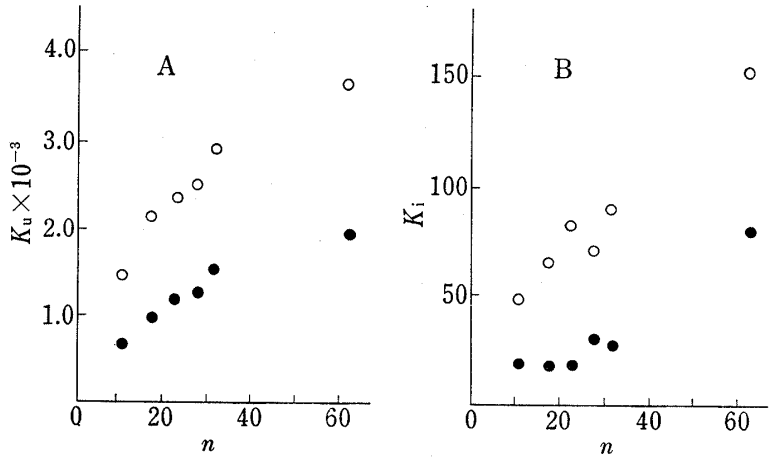

Fig. 6. Relationship between K and n at 25°

A) K_u at pH 2.04, B) K_i at pH 7.32.
○, PGE_1, ●, $PGF_{2\alpha}$.

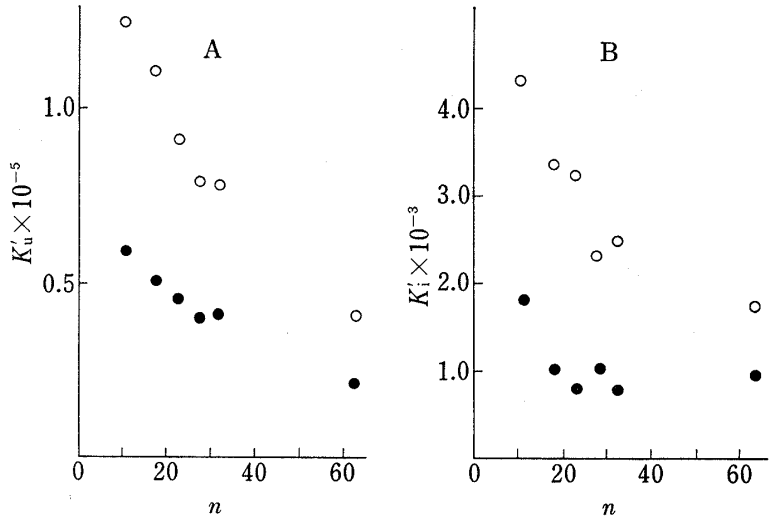

Fig. 7. Relationship between K' and n at 25°

A) K_u' at pH 2.04, B) K_i' at pH 7.32.
○, PGE_1, ●, $PGF_{2\alpha}$.

Micelles formed by nonionic surfactants with long oxyethylene chains are nearly spehrical.[16] A cross-sectional view of the micellar pseudophase has been assumed to range from the hydrophobic interior core to increasingly hydrated oxyethylene chains forming the outer layer of the micelle and to the micellar surface surrounded by water. Therefore, the volume fraction of the hydrophobic core region in the micellar phase would decrease with increasing hydrophilic chain length even if the aggregation number remained almost constant. In fact, the reduction of the aggregation number would cause a further reduction in the volume fraction of the core portion. If the interior core region was a predominant site of interaction for a drug, the P value would naturally decrease with increasing oxyethylene chain length. The decrease of the P value for PGE_1 and $PGF_{2\alpha}$ with the number of oxyethylene units suggests that the primary site of interaction is located in the core portion.

16) D.I.D. El Eini, B.W. Barry, and C.T. Rhodes, *J. Colloid Interface Sci.*, **54**, 348 (1976).

NII-Electronic Library Service

In the case of cetomacrogol ($n=30$), which carries a longer hydrocarbon chain than lauromacrogol, the P_u and P_i values of PGE_1 were 2956 and 224, respectively.[17] These values are much larger than those of PLE28 when the oxyethylene chain length is comparable. This supports the view that PGE_1 is mainly accommodated in the hydrophobic core region.

It should, however, be noted that in the case the unionized species, the P dependency on n for $PGF_{2\alpha}$ was less oblique than that of PGE_1, namely the slope was dependent on the lipophilic character of the drugs. In the case of the ionized species, both slopes were little affected by n above about $n=30$. These results suggest that although the ratio of the drug in the two parts of the micelle cannot be determined with certainty, the drugs, regardless of the species, are also accommodated to some extent in the oxyethylene portion.

The K value, defined on a molar basis as the magnitude of interaction free from the formation of the micellar phase, increases with increasing oxyethylene chain length. This appears to indicate that the longer the oxyethylene chain length, the more binding occurs. Thus, there seems to be an inconsistency between the trends of K and K'. However, this can be explained as follows. On a per micelle basis, the reduction of the aggregation number with hydrophilic chain length, *i. e.*, an increase of the micelle concentration, more than counteracts the increase of the K value, leading to the observed decrease in the K' values with increasing oxyethylene chain length. This is consistent with the trends of the P values, for which similar considerations should be applicable.

Gouda *et al.* reported that the partition coefficients of barbiturates between polyoxyethylene stearate micelles and water increase with increasing oxyethylene chain length, as do the values of moles of drug per mole of sufractant.[18] According to Barry and El Eini, the solubilizing efficiencies of non-polar steroids into the increasingly hydrophilic micellar environments of polyoxyethylated cetyl alcohols decreases, and the partition coefficient and the value of amount of drug per micelle were useful measures of the interaction magnitude.[19] Thus, the measures of the degree of interaction are desired to be closely related to the changes of micelle characteristics, *i. e.*, to the amounts of drugs in the hydrophobic and hydrophilic regions available for drug binding.

17) S. Oguri, T. Yotsuyanagi, and K. Ikeda, to be published.
18) M. Wafik Gouda, A.A. Ismail, and M.M. Motawi, *J. Pharm. Sci.*, **59**, 1402 (1070).
19) B.W. Barry and D.I.D. El. Eini, *J. Pharm. Pharmacol.*, **28**, 210 (1976).

NII-Electronic Library Service