International Journal of Pharmaceutics, 1 (1978) 281–297

QUANTITATIVE ASSESSMENT OF THE NEGATIVE CATALYTIC EFFECTS OF A CATIONIC SURFACTANT MYRISTYL-γ-PICOLINIUM CHLORIDE ON THE SPECIFIC-ACID CATALYZED EPIMERIZATION OF 15(S)-15-METHYL PROSTAGLANDIN $F_{2\alpha}$

M.J. CHO and M.A. ALLEN

Pharmacy Research Unit, The Upjohn Company, Kalamazoo, Mich. 49001 (U.S.A.)

(Received December 12th, 1977)
(Accepted April 1st, 1978)

SUMMARY

The specific-acid catalyzed epimerization of 15(S)-15-methyl $PGF_{2\alpha}$ was found to be significantly inhibited in the presence of a cationic surfactant, myristyl-γ-picolinium chloride, at pH 2.5 and 25°C. The micellar inhibition observed is attributed to electrostatic repulsion between hydronium ion and the cationic polar heads of the micellar phase. As expected, at a given concentration of prostaglandin, the observed rate decreased as the surfactant concentration increased. When the concentration of prostaglandin was in the order of 7×10^{-2} mg/ml (2×10^{-7} M), the observed epimerization rate in a 1.0% surfactant solution was found to be approximately 120 times slower than that observed in the absence of surfactant. A quantitative analysis showed that the epimerization rate constant in the micellar phase is in the order of 6×10^{-7} s^{-1} (cf. 2×10^{-4} s^{-1} in the absence of the surfactant).

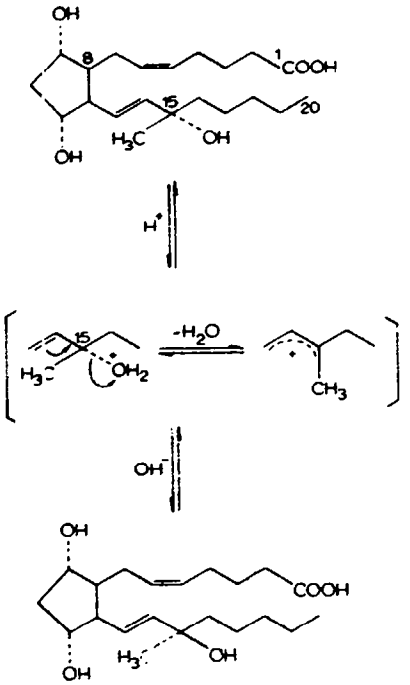
However, the extent of micellar inhibition decreased as the initial concentration of prostaglandin increased in a series of solutions containing a constant amount of the surfactant. In order to quantitatively interpret this result, the apparent partition coefficient (ψ_m) of the prostaglandin between the micellar phase and the aqueous bulk phase was determined using a partitioning technique. Heterogeneity of the binding sites was detected in the analysis of ψ_m using the Scatchard equation.

The primary binding sites are postulated to occur by a short penetration of prostaglandin molecules in the palisade layer. The secondary binding sites can be provided by either a simple adsorption process of prostaglandin onto the surface of micelles or the formation of mixed micelles. Although the primary binding sites show approximately 75-fold greater affinity towards the prostaglandin than the secondary binding sites, the number of available sites of the latter is approximately 25 times greater than that of the former. It is proposed that the Scatchard equation be used in the quantitative analysis of the effects of the substrate concentration at a constant surfactant concentration upon the observed micellar catalysis, a subject which has been unjustifiably neglected in the past.

INTRODUCTION

Although enzymatic oxidation of the allylic hydroxyl group at C-15 of the naturally occurring prostaglandins is the primary mode of metabolic deactivation, it can be significantly retarded when a bulky substituent is attached to either the C-15 or C-16 position. Thus, 15-methyl (Yankee et al., 1974) and 16,16-dimethyl (Magerlein et al., 1973) prostaglandin analogs are generally more potent and exhibit a longer duration of action than the parent prostaglandins. However, by introducing a methyl group at C-15, the tertiary C-15 becomes very susceptible to carbonium ion formation (March, 1968). The process is particularly facile because the resulting carbonium ion has a direct resonance stabilization interaction with the 13,14-double bond. In aqueous solutions, water molecules can then attack the carbonium ion from either direction of the plane of the carbonium ion to form a mixture of R- and S-epimers.

For instance, as shown below, 15(S)-15-methyl-$PGF_{2\alpha}$, one of the most potent abortifacient prostaglandins (Karim, 1975), reversibly undergoes epimerization at C-15 to form 15(R)-epimer. The latter was found to have only minimal biological activity. As a part of our concerted efforts to inhibit this major decomposition reaction of 15-methyl prostaglandins in aqueous media, we investigated the possible utilization of negative micellar catalysis.



Specific-acid catalyzed epimerization of 15(S)-15-methyl $PGF_{2\alpha}$.

Micelle-catalyzed organic reactions in aqueous media have been extensively studied as model systems of enzymatic reactions, and accelerations of reactions in the order of 10^2 or 10^3 by micellar catalysis has been frequently reported (Tanford, 1973; Bunton, 1973; Gordes, 1973; Fendler and Fendler, 1975; Piszkiewicz, 1977). In sharp contrast, one can

find only a few quantitative studies on the inhibitory or negative catalytic effects of micelles on chemical reactions, although it has profound implication in the stabilization of chemically unstable drug compounds in aqueous pharmaceutical preparations. In a micellar solution, the rate of an organic reaction can be remarkably different from that observed in the absence of surfactant, basically because the reaction rate in the micellar phase is different from that in the bulk solution phase and because substantial amounts of reactants can be distributed into the micellar phase. The observed rate constant (k_{obs}) is then;

$$k_{obs} = k_0(1 - F_m) + k_m F_m \quad (1)$$

where k_m and k_0 are the rate constants of a given reaction in the micellar phase and in the bulk solution, respectively, and F_m is the fraction of the reactants present in the micellar phase. For a given micellar system, k_m is constant at a given temperature. However, F_m varies depending on the total concentrations of surfactant and drug. At a given drug concentration, k_{obs} is expected to decrease as the total surfactant concentration increases so long as $k_m < k_0$, simply because a larger micellar *phase volume* is expected at a higher concentration of the surfactant. On the other hand, if the simple partition model described above is applied, assuming that the partition coefficient of a drug compound is independent of the total drug concentration (at least in a dilute solution), one can expect k_{obs} to be independent of drug concentration at any given surfactant concentration.

From a mechanistic point of view, one can collectively attribute the difference between k_m and k_0 to proximity, electrostatic, and medium effects (Bender, 1971), although the individual contribution of each of these effects cannot be easily assessed. Intuitively, however, one can expect a most pronounced alteration in reaction rate when the reactants involved are charged species and the surfactant employed is ionic. The specific-acid catalyzed epimerization of 15(S)-15-methyl $PGF_{2\alpha}$ requires H_3O^+, and the effective concentration of H_3O^+ can be extremely low in the vicinity of the prostaglandin molecules present in the micellar phase. Also, the formation of a carbonium ion at C-15, most likely the rate-determining step in epimerization, inevitably involves a polar transition state. Although this transition state is mechanistically considered less polar (i.e. involves the dispersion of a formal charge) than the ground state of the reaction, carbonium ion formation in such a non-polar environment as in the micellar phase should be extremely unfavorable. Discussed in the present report are the inhibitory effects of a cationic surfactant, myristyl-γ-picolinium chloride, on the acid-catalyzed epimerization reaction of 15-methyl prostaglandins. As will become evident, the reaction not only serves as an ideal model system for a quantitative study of micellar inhibition but also bears practical significance in that it is the major decomposition reaction of the prostaglandin in aqueous media.

MATERIALS AND METHODS

Materials

15(S)-15-methyl $PGF_{2\alpha}$ free acid ($C_{21}H_{36}O_5$; MW = 368.52) was obtained from an aqueous solution of its tris(hydroxymethyl)aminomethane (THAM) salt (Upjohn Co.) by extraction with ethyl acetate after the pH of the solution was adjusted to approximately 4.5 with a diluted formic acid solution. After the ethyl acetate layer was dried with

anhydrous sodium sulfate, the solvent was thoroughly evaporated under high vacuum for at least 5 h. A viscous pale-yellow liquid was obtained. Myristyl-γ-picolinium chloride [CH_3–$C_5H_4N^+$–$(CH_2)_{13}CH_3Cl^-$] (MW = 325.96) was used as received without purification (Quatresin, Upjohn Co.). The derivatizing agents for HPLC analysis of the prostaglandin, *p*-nitrophenacyl bromide and diisopropylethyl amine, were obtained from The Aldrich Co. The former was recrystallized from acetone and *n*-hexane, and the latter was distilled under high vacuum at 70–75°C. Stock solutions of both reagents were prepared in acetonitrile. Organic solvents were all of analytical grade (Burdick and Jackson, distilled in glass). *p*-Nitrophenacyl ester of desoxycholic acid, an internal standard used in high performance liquid chromatograph (HPLC) work, was kindly provided by Dr. W. Morozowich of The Upjohn Company.

High performance LC columns obtained from E. Merck (Hibar LiChrosorb Si 60; 3.0 mm ID × 25 cm L) and from DuPont (Forbax Sil; 4.6 mm ID × 25 cm L) were used throughout the present study in a DuPont Model 830 HPLC equipped with a Hewlett-Packard Model 3380A integrator and a DuPont Model 837 variable-wavelength spectrophotometer. A Beckman Century SS-1 pH meter was used with Beckman electrodes throughout the studies. The CMC (critical micellar concentration) of the surfactant was determined by an automated drop volume apparatus (Rowe, 1972).

Kinetic experiments

At proper time intervals, an aliquot of 4.0 ml was transferred from a sample solution of 6.44×10^{-2} mg/ml in 15-methyl $PGF_{2\alpha}$ to a 15-ml centrifuge tube containing 5.0 ml of ethyl acetate and 25 μl of 7.290 mg/ml *p*-nitrophenacyl ester of desoxycholic acid in acetonitrile. After vigorous shaking, approximately 4 ml of the ethyl acetate layer was withdrawn and evaporated under nitrogen stream at room temperature. *p*-Nitrophenacylation was then carried out as described by Morozowich (1975), by adding to the above residue 120 μl of diisopropylethylamine (10 μl/ml) and 170 μl of *p*-nitrophenacyl bromide (25 mg/ml), both in acetonitrile. After approximately 30 min at room temperature, the reaction mixture was subjected to HPLC analysis. When the concentration of total prostaglandins was other than 6.44×10^{-2} mg/ml, varying amounts of the derivatization reagents proportional to the amounts listed above were used.

Throughout the experiments, the kinetic sample solutions were prepared by diluting a stock solution of 15(S)-15-methyl $PGF_{2\alpha}$ in acetonitrile at least 100-fold in a desired buffer solution. The experimental pH was adjusted prior to each kinetic run and measured once again at the end. In general, the pH drift was less than 0.05 pH units during an experiment. It was also found that the presence of cationic surfactant did not affect the pH significantly under the present experimental conditions. Temperature of the epimerization sample solutions was kept at 25 ± 0.02°C in a water bath (Neslab Instruments).

For the reversible $S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} R$ reaction, where S and R represent 15(S)- and 15(R)-epimers of 15-methyl $PGF_{2\alpha}$, the first-order rate constants, k_f and k_r, can be obtained from Eqns. 2 and 3 (Frost and Pearson, 1961):

$$S_e \ln\left(\frac{S_0 - S_e}{S - S_e}\right) = S_0 k_r t \qquad (2)$$

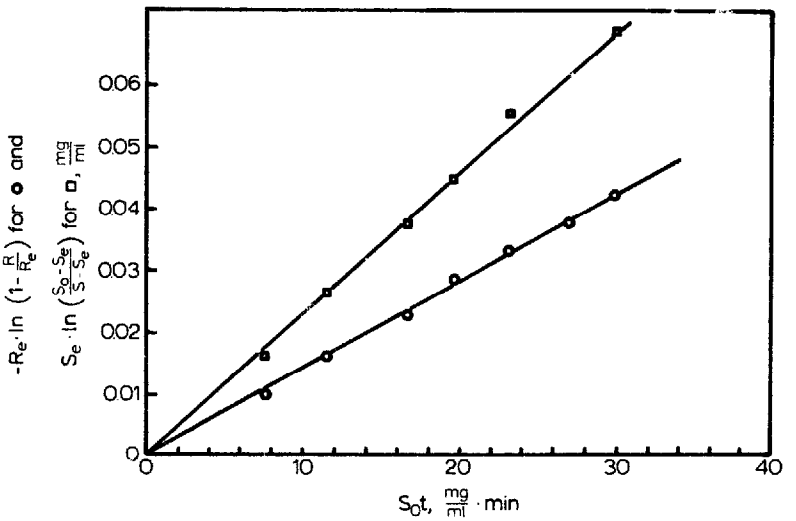


Fig. 1. Calculations of the forward (○) and the reverse (□) epimerization rate constants of 15(S)-15-methyl $PGF_{2\alpha}$ in a 0.2% myristyl-γ-picolinium chloride solution at pH 2.50 and 25°C using Eqns. 2 and 3. The initial prostaglandin concentration (S_0) was 0.0644 mg/ml (1.75×10^{-7} M).

$$-R_e \ln\left(1 - \frac{R}{R_e}\right) = S_0 k_f t \tag{3}$$

where S_0, S and S_e indicate the concentrations of the (S)-epimer at t = 0, at a given time, t, and at equilibrium, respectively; similarly R and R_e indicate the concentrations of (R)-epimer at a given time and at equilibrium, respectively. Typical plots of Eqns. 2 and 3 are exemplified in Fig. 1 for the reaction system containing 0.20% of the surfactant.

HPLC analysis of prostaglandins

A reaction mixture of *p*-nitrophenacylation of prostaglandins in acetonitrile was directly injected on an HPLC column under the following conditions: mobile phase = methylene chloride (96.5 volume) + methanol (3.5 vol.); pressure = 1500 psi; wavelength of detection = 263 nm; flow rate = about 2.30 ml/min with a Zorbax Sil column. The HPLC procedure was used in determining prostaglandin concentrations in the partition coefficient and the solubility measurement as well as the epimerization kinetics. A series of typical HPLC separations of 15(S)- and 15(R)-epimers as *p*-nitrophenacyl esters are shown in Fig. 2. As shown in Fig. 2C, at equilibrium, approximately 6% of the total prostaglandins was decomposed to side products, for which chemical structures have not yet been established (see peaks at 4.28 and 4.99 min.). When the first 2 ~ 3 half-lives were used in calculating k_f and k_r of the epimerization reaction, these side reactions were found to be negligible. Under this condition, it was not necessary to use the internal standard (i.e. normalization technique served as the purpose of analysis). When the internal standard was used (as in cases of partition and solubility experiments), the total concentrations of prostaglandins were calculated using a calibration curve constructed from a series of synthetic mixtures of known prostaglandin concentrations. The HPLC analysis described above was not affected by the presence of surfactant, presumably

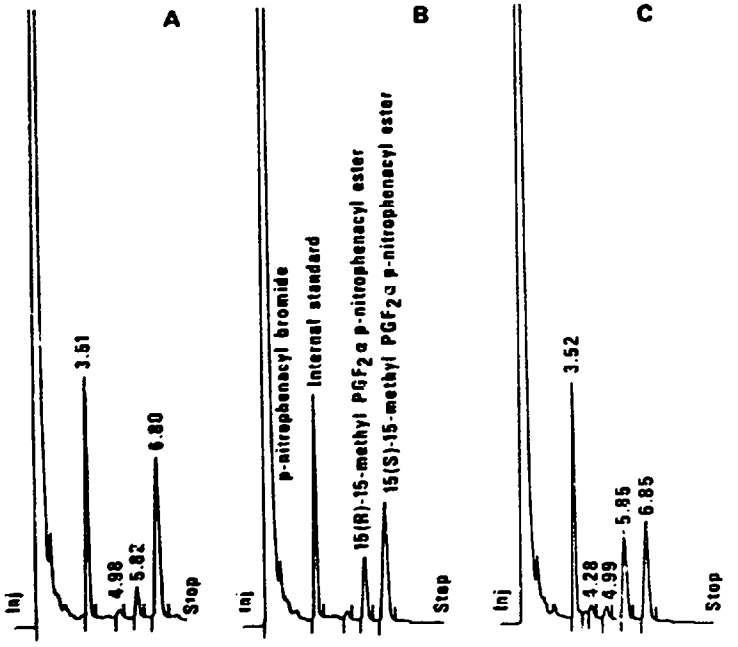


Fig. 2. Typical HPLC analyses of 15(S)- and 15(R)-epimers of 15-methyl $PGF_{2\alpha}$ as *p*-nitrophenacyl esters. Samples were from an epimerization reaction mixture at pH = 3.00 and 25°C: A, 30 min-; B, 90 min-; C, 180 min-old samples, respectively.

because the surfactant did not partition into the ethyl acetate layer during extraction of the prostaglandin (see Kinetic experiments).

Partition experiments

The partition coefficient (ψ_m) of the prostaglandin between the micellar phase and the aqueous bulk phase was measured as described below. Note that ψ_m is expressed in terms of mass rather than concentration. The partition coefficient in terms of concentration could not be measured, because it was not possible to estimate the micellar phase volume. A 1 : 1 (by volume) mixture of ethyl acetate and *n*-hexane and a pH 2.50 solution of HCl were used in measuring ψ_m.

First, in a series of separate experiments, the apparent partition coefficient (PC′) was accurately measured in the absence of surfactant. Then, in the presence of surfactant in the HCl solution, the prostaglandin concentration at equilibrium in the aqueous *bulk* phase was determined through the PC′ value and the prostaglandin concentration found in the organic-solvent layer. Finally, the amount of the prostaglandin present in the micellar phase was obtained by subtracting the amount of the prostaglandin found in the aqueous *bulk* phase from the total recovery in the aqueous layer (including the micellar phase). The overall calculation procedure is shown in Tables 1–3. One assumption made in the calculation was that the total volume of aqueous layer remained constant even when different amounts of the surfactant were added. From the definitions of F_m (see Eqn. 1) and ψ_m, it can be shown that

$$F_m = \frac{\psi_m}{1 + \psi_m} \tag{4}$$

Throughout the experiments, 15-ml centrifuge tubes containing the prostaglandin and the surfactant in a binary phase system were shaken in a water bath at 25°C for at least 24 h. Two series of experiments were carried out; one with varying amounts of the surfactant at a constant concentration of total prostaglandin (Table 1) and the other with varying amounts of total prostaglandin at two constant surfactant concentrations, 0.20% (Table 2) and 0.50% (Table 3). In the experiments with a series of 0.50% surfactant solutions, a surfactant solution which already had been saturated with the prostaglandin was properly diluted in a 5.0% solution in a pH 2.50 HCl solution to cover a maximum range of the total prostaglandin.

Since the initial concentration (or total amount) of the prostaglandin was accurately known in all samples, it was necessary to analyze that of only one phase. For the sake of convenience, the organic-solvent layer was analyzed using the HPLC procedure described above. During the partition equilibration, epimerization was found to take place substantially. However, one can assume that 15(R)- and 15(S)-epimers of 15-methyl $PGF_{2\alpha}$ have an identical ψ_m value.

Determination of the prostaglandin solubility as a function of surfactant concentration

Into a series of 10 ml centrifuge tubes, excess 15(S)-15-methyl $PGF_{2\alpha}$ and 5.0 ml of pH 2.5 HCl solutions containing varying amounts of the surfactant (myristyl-γ-picolinium chloride) were added. Teflon-lined caps were tightly wrapped with Parafilm (American Can Co.) and the tubes were shaken for at least 24 h at 25°C in a water bath. At solubility equilibrium, samples were centrifuged. A 2.0 ml aliquot of the supernatant was subsequently extracted with 5.0 ml ethyl acetate containing a proper amount of the internal standard. After evaporation of the solvent, the residual prostaglandin was derivatized and subject to HPLC analysis as described above.

Measurement of the critical micellar concentration (CMC) of myristyl-γ-picolinium chloride

By successive dilutions of two stock solutions of the cationic surfactant (2.0 and 0.1%), a series of sample solutions were prepared in double distilled water with the surfactant concentration ranging from 3.125×10^{-3} to 2.0%. An automated drop volume apparatus (Rowe, 1972) was then used to measure the surface tension of each solution. From a plot of the measured surface tension against the surfactant concentration, one can obtain the CMC.

RESULTS AND DISCUSSION

For a pharmaceutical formulator, perhaps one of the most relevant questions concerning negative micellar catalysis is how the observed rate constant (k_{obs}) of a hydrolytic reaction varies with the drug and the surfactant concentrations. At a given drug concentration, k_{obs} should be independent of the surfactant concentration when the latter is below the CMC of a surfactant, unless the monomer of the surfactant undergoes a molecular interaction with the drug compound to alter the reaction rate. When the concentration of the surfactant exceeds the CMC, the drug molecules begin to partition into the micellar phase formed. Further increase in the surfactant concentration would provide a

larger micellar phase volume to result in a more profound change in k_{obs}. When the drug concentration is also altered, the finding reported by Mitchell (1963) appears to be the most appropriate generalization advanced thus far in the literature. He observed that the degree of inhibition of alkaline hydrolysis of *n*-propyl benzoate by a non-ionic surfactant, Cetomacragol 1000, depends critically on the so-called saturation ratio rather than the absolute concentrations of the ester or the surfactant; a faster hydrolysis rate was reported at a higher saturation ratio. The saturation ratio was simply defined as the ratio of the total drug concentration to its solubility at a given surfactant concentration.

If this observation holds true for hydrolytic reactions in general, then a maximum degree of stabilization of a given drug will be obtained in an aqueous solution of the drug which is very diluted in terms of the drug but concentrated with respect to surfactant. The present report generally agrees with this presupposition.

Critical micellar concentration (CMC) of myristyl-γ-picolinium chloride

As shown in Fig. 3, the surface tension of the surfactant solution at 25°C levels off approximately at 0.125% (3.83×10^{-3} M); no minimum due to possible impurities was detected. This CMC value is comparable to those of other pyridinium halide surfactants (e.g. dodecyl pyridinium bromide; Mukerjee and Mysels, 1971).

Solubility and apparent partition coefficient (ψ_m) of 15-methyl $PGF_{2\alpha}$

As shown in Fig. 4, the apparent solubility of 15-methyl $PGF_{2\alpha}$ steadily increases as the concentration of myristyl-γ-picolinium chloride increases. It is noteworthy that there is no sharp break at the CMC of the surfactant, 0.125%. This finding, together with the gradual increase in ψ_m and F_m (see Table 1) even at a surfactant concentration far below the CMC, appears to indicate that there exist some kinds of molecular interaction between monomeric surfactant molecules and the prostaglandins. If one assumes that the concentration of monomeric surfactant molecules remains essentially constant (equal to the CMC) with the excess molecules forming micelles (Mukerjee and Mysels, 1971) and

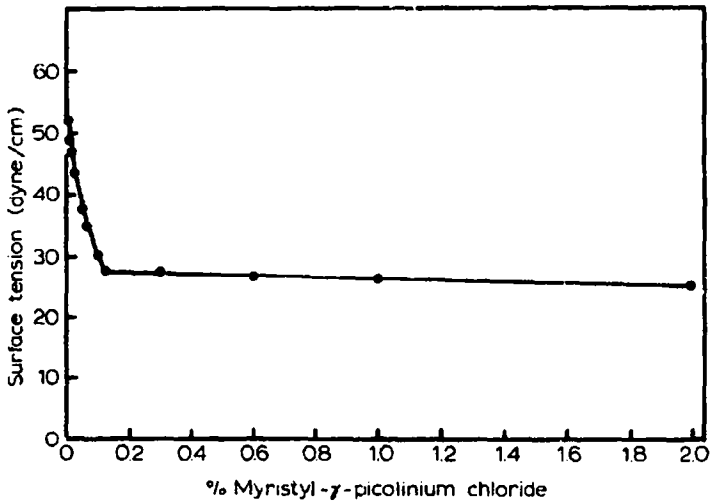


Fig. 3. Determination of the critical micellar concentration (CMC) of myristyl-γ-picolinium chloride in double distilled water at 25°C.

TABLE 1

ESTIMATION OF ψ_m (PARTITION COEFFICIENT OF 15-METHYL $PGF_{2\alpha}$ BETWEEN THE MICELLAR PHASE AND THE AQUEOUS BULK PHASE) AND F_m (FRACTION OF 15-METHYL $PGF_{2\alpha}$ PRESENT IN THE MICELLAR PHASE) AT pH 2.50 AND 25°C, AS A FUNCTION OF THE SURFACTANT CONCENTRATION AT A CONSTANT TOTAL AMOUNT (0.322 mg) OF THE PROSTAGLANDIN IN THE SYSTEM.

A = Total volume (ml) of the aqueous phase; B = concentration of the prostaglandin (mg/ml) found in the organic later at the partition equilibrium; C = B × 5.0 = amount (mg) of the prostaglandin found in the organic layer (5.0 ml); D = 0.322 − C = amount (mg) present in the aqueous layer; E = B/2.204 = the prostaglandin concentration (mg/ml) present in the aqueous *bulk* phase, where 2.204 is the apparent partition coefficient in the absence of the surfactant; F = E × A = mg prostaglandin present in the aqueous *bulk* phase; G = mg prostaglandin present in the micellar phase = D − F; ψ_m = G/F; F_m = see Eqn. 4.

Percent surfactant	A	B	C	D	E	F	G	ψ_m	F_m
0	5.00	0.0443	0.222	0.101	–	–	–	0	0
0.010	5.005	0.0426	0.213	0.109	0.0193	0.0966	0.0124	0.128	0.113
0.050	5.025	0.0410	0.205	0.117	0.0186	0.0935	0.0235	0.251	0.201
0.099	5.05	0.0318	0.159	0.163	0.0144	0.0729	0.090	1.236	0.553
0.099	5.05	0.0325	0.163	0.160	0.0147	0.0745	0.085	1.141	0.533
0.148	5.075	0.0258	0.129	0.193	0.0117	0.0593	0.134	2.255	0.693
0.196	5.10	0.0232	0.116	0.206	0.0105	0.0537	0.196	3.641	0.785
0.196	5.10	0.0229	0.115	0.208	0.0104	0.0530	0.155	2.915	0.745
0.291	5.15	0.0163	0.082	0.241	0.0074	0.0381	0.202	5.312	0.842
0.291	5.15	0.0173	0.089	0.234	0.0080	0.0414	0.192	4.640	0.823
0.385	5.20	0.0115	0.058	0.253	0.0052	0.0271	0.226	8.336	0.893
0.385	5.20	0.0132	0.066	0.256	0.0060	0.0311	0.225	7.232	0.879
0.566	5.30	0.0109	0.055	0.268	0.0049	0.0262	0.241	9.210	0.902
0.654	5.35	0.0087	0.044	0.279	0.0040	0.0211	0.257	12.187	0.924
0.740	5.40	0.0077	0.039	0.284	0.0035	0.0189	0.265	14.030	0.933
0.826	5.45	0.0062	0.031	0.291	0.0028	0.0153	0.276	17.980	0.947
0.909	5.50	0.0057	0.029	0.293	0.0026	0.0143	0.279	19.550	0.951

that the molecular interactions between the prostaglandin and the monomeric surfactant molecules bring about a linear increase in the apparent solubility of the drug (Higuchi and Connors, 1965) then, as shown in Fig. 4, the values of ψ_m and F_m can be approximated from the solubility data. The ψ_m and F_m values thus obtained are in fair agreement with the extrapolation of those obtained by the partition technique (Table 2). The agreement between ψ_m values determined by the partition experiments and the solubility study is purely accidental, because the former was calculated in terms of concentration. For a series of 0.5% surfactant solutions, the partition experiments were carried out with a 0.5% solution which had already been saturated with the prostaglandin. In this way it was possible to cover a wide range of the total prostaglandin (Table 3). As will be seen later, the significant heterogeneity of binding sites can be detected only when a sufficient amount of prostaglandin is present in the system.

The values of ψ_m and F_m reported in Tables 1–3 are admittedly subject to large

TABLE 2

ESTIMATION OF ψ_m AND F_m AT pH 2.50 and 25°C, AS A FUNCTION OF THE TOTAL PROSTAGLANDIN AT A CONSTANT SURFACTANT CONCENTRATION (0.20%)

H = Initial concentration (mg/ml) of 15-methyl $PGF_{2\alpha}$; I = H × 5.10 = total amount (mg) of the prostaglandin present in the system (volume of the aqueous layer = 5.10 ml); the rest of the legends are identical to those in Table 1.

H	I	B	C	D	E	F	G	ψ_m	F_m
0.0551	0.281	0.0245	0.123	0.159	0.0111	0.057	0.102	1.795	0.642
0.0631	0.322	0.0232	0.116	0.206	0.0105	0.0537	0.196	3.641	0.785 [a]
0.0531	0.322	0.0229	0.115	0.208	0.0104	0.0530	0.155	2.915	0.745 [a]
0.1102	0.562	0.0467	0.234	0.329	0.0212	0.108	0.220	2.039	0.671
0.5512	2.810	0.247	1.235	1.575	0.1121	0.572	1.003	1.755	0.637
1.1022	5.621	0.548	2.740	2.881	0.2486	1.268	1.613	1.272	0.560
1.6529	8.430	0.923	4.615	3.815	0.4188	2.136	1.679	0.786	0.440
2.2043	11.242	1.162	5.810	5.432	0.5272	2.689	2.743	1.020	0.505
2.7555	14.053	1.560	7.800	6.253	0.7078	3.610	2.643	0.732	0.423
4.21	21.471	--	--	--	--	13.209	8.262	0.625 [b]	0.385 [b]

[a] From Table 1 (data at the surfactant concentration 0.196%).
[b] From solubility data (see Fig. 3 and the text).

errors. The major source of error is due to the fact that the concentration of the prostaglandin was not experimentally determined in both organic and aqueous layers at the partition equilibrium. Thus it would be kept in mind that the errors propagate as the calculation proceeds in the tables. Nevertheless, a few significant observations can be made. First, as expected, the values of ψ_m and F_m increase as the surfactant concentration increases when a given amount of the prostaglandin is present in the partition system (Table 1). For instance, in a 0.909% surfactant solution, approximately 95% of 0.322 mg of prostaglandin exists inside the micellar phase (as can be seen later, the word *inside* is a bona fide expression in this context).

Second, it is interesting to examine how the values of ψ_m and F_m vary with the amount of prostaglandin present in the systems containing a constant amount of the surfactant (Tables 2 and 3). As more prostaglandin is added, the most favorable binding sites in the micellar phase will soon be saturated with prostaglandin. Further drug molecules have to find other binding sites with less affinity towards them. Finally, a dynamic distribution equilibrium is forced by the solubility limit of the drug in the aqueous bulk phase. This qualitative picture of the drug distribution process implies a heterogeneity of the binding sites of the micellar phase towards a given organic compound and is analogous to the protein–drug binding process.

A general discussion of the principles and concepts fundamental to the binding capacity of proteins with various substances may be found in the papers by Scatchard et al. (1954), Edsall and Wyman (1958), Foster (1960), Tanford (1965), and Weber (1965). The mathematical theory associated with such studies is discussed in detail by Krüger-Thimer et al. (1964), Hart (1965), Sandberg et al. (1966), and Rosenthal (1967). Briefly, the so-called Schatchard equation (Eqn. 5) is applicable to the present multiple equilibria

TABLE 3

ESTIMATION OF ψ_m AND F_m AT pH 2.50 AND 25°C AS A FUNCTION OF THE TOTAL PROSTAGLANDIN AT A CONSTANT SURFACTANT CONCENTRATION (0.50%).

H = Initial concentration (mg/ml) of 15-methyl $PGF_{2\alpha}$; I = H × 5.10 = total amount (mg) of the prostaglandin present in the system (volume of the aqueous layer = 5.10 ml); the rest of the legends are identical to those in Table 1.

H	I	B	C	D	E	F	G	ψ_m	F_m
0.0443	0.2326	0.0089	0.0445	0.1881	0.0040	0.0212	0.167	7.87	0.887
0.0613	0.3218	0.0130	0.0650	0.2568	0.0059	0.0310	0.226	7.29	0.879
0.0797	0.4184	0.0161	0.0805	0.3379	0.0073	0.0384	0.300	7.81	0.887
0.0920	0.4830	0.0176	0.0880	0.3950	0.0080	0.0419	0.353	8.42	0.894
0.104	0.5460	0.0205	0.1025	0.4435	0.0093	0.0488	0.395	8.08	0.890
0.107	0.5618	0.0245	0.1275	0.4393	0.0111	0.0584	0.381	6.53	0.867
0.123	0.6458	0.0247	0.1235	0.5223	0.0112	0.0588	0.464	7.88	0.887
0.141	0.7403	0.0284	0.1420	0.5983	0.0129	0.0677	0.531	7.84	0.887
0.153	0.8033	0.0299	0.1495	0.6538	0.0136	0.0712	0.583	8.18	0.891
0.268	1.407	0.0741	0.3705	1.0365	0.0336	0.1765	0.860	4.87	0.830
0.536	2.814	0.1354	0.7700	2.044	0.0614	0.3225	1.722	5.34	0.842
1.070	5.618	0.2970	1.485	4.133	0.1348	0.7075	3.426	4.84	0.829
2.140	11.24	0.6219	3.109	8.126	0.2822	1.481	6.644	4.49	0.818
3.212	16.86	1.013	5.064	11.800	0.4595	2.412	9.387	3.89	0.796
4.280	22.47	1.489	7.446	15.025	0.6756	3.547	11.483	3.24	0.764
5.350	28.09	1.743	8.717	19.371	0.7910	4.153	15.218	3.67	0.786
6.420	33.71	2.574	12.868	20.838	1.168	6.130	14.707	2.40	0.706
7.490	39.32	2.794	13.972	25.351	1.268	6.656	18.695	2.81	0.737
8.570	44.99	3.297	16.486	28.508	1.496	7.854	20.924	2.66	0.727
9.640	50.61	3.528	17.639	32.972	1.601	8.403	24.568	2.92	0.745

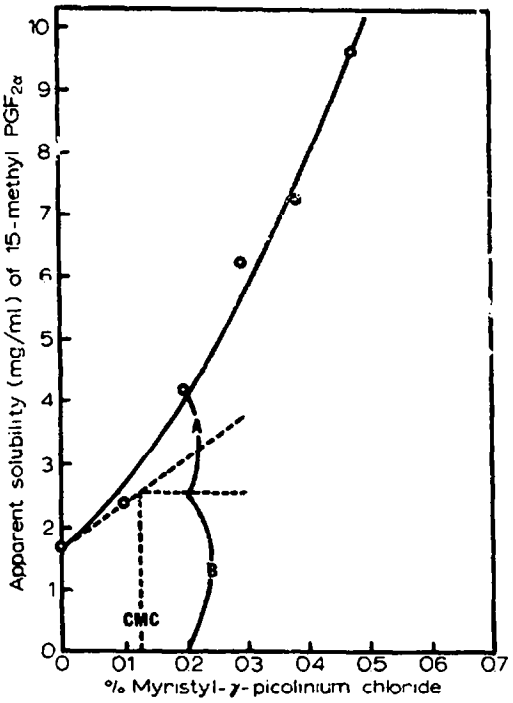


Fig. 4. Apparent solubility of 15-methyl $PGF_{2\alpha}$ as a function of the surfactant concentration at pH 2.50 and 25°C. The ψ_m value (see the text) was determined by A/B at 0.20% surfactant (Table 2).

of the drug distribution between micellar phase and aqueous bulk phase as below:

$$\frac{(D_b)}{(M_T)(D_f)} = n \cdot K - K \frac{(D_b)}{(M_T)} \tag{5}$$

Where D_b and D_f represent the *concentrations* of the drug bound to the micellar phase and the unassociated drug in the aqueous bulk phase, and n and K represent the number of a given type of binding sites present in one micellar aggregate and K is the intrinsic association constant of the drug with one of that particular binding site in the micellar phase.

For the data given in Tables 2 and 3, the concentration of the micellar aggregate (M_T) = C/N, where C is the total concentration of the surfactant and N is the aggregation number, remains constant. When Eqn. 5 is modified using *mass* terms rather than concentration terms, Eqn. 6 can be obtained:

$$\psi_m = n \cdot K \cdot M_T - K \cdot D_b \tag{6}$$

where the intrinsic association constant K is expressed in terms of a reciprocal of mass. The data obtained for a series of 0.5% surfactant solutions (Table 3) were plotted following Eqn. 6 and are shown in Fig. 5. If one follows a graphical way to present the binding parameters (Rosenthal, 1967), the partition data obtained from a series of 0.5% surfactant solutions can be interpreted by assuming two different binding sites, as indicated by two straight lines in Fig. 5.

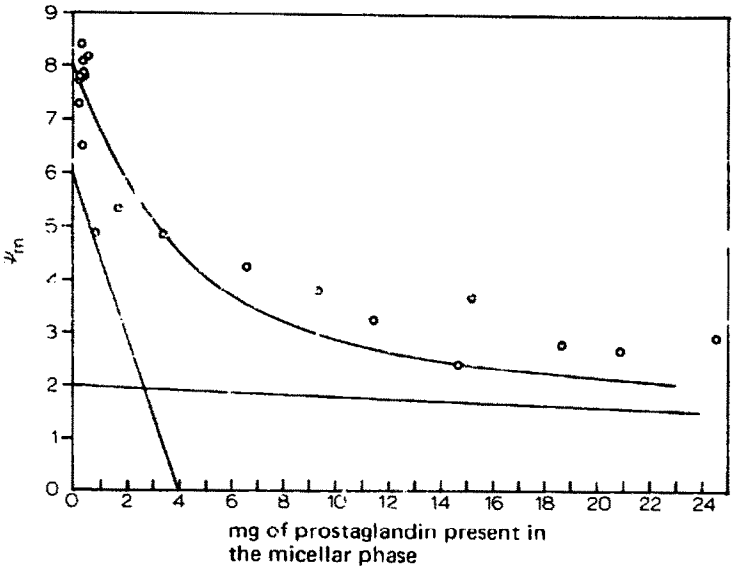


Fig. 5. Modified Scatchard plots for the partition of 15-methyl $PGF_{2\alpha}$ between the micellar phase and the aqueous bulk phase at pH 2.50 and 25°C in a series of 5.0% myristyl-γ-picolinium chloride solutions. The binding curve was obtained from the straight lines using a graphical method developed by Rosenthal (1967).

From the ratio of the slopes (see Eqn. 6), one can state that the affinity of the primary binding sites is approximately 75 times greater than that of the secondary binding sites. The x-axis intercept, nM_T (see Eqn. 6), indicates the total number of moles (not mole/l) of a given *type* of binding sites present in the system. The ratio of nM_T for the secondary to the primary binding sites was found to be approximately 25 : 1. These quantitative analyses can be summarized as follows. The micellar phase formed by a cationic surfactant, myristyl-γ-picolinium chloride, provides two different binding sites for 15-methyl $PGF_{2\alpha}$. Although the primary binding sites show approximately 75-fold greater affinity towards the prostaglandin than the secondary binding site, the number of available sites of the latter is approximately 25 times greater than that of the former.

Negative catalytic effects of surfactant on the epimerization of 15-methyl $PGF_{2\alpha}$

A series of preliminary kinetic experiments without the surfactant were performed to examine the pH-dependence of the epimerization reaction. Results clearly showed that at pH 2.5 the reaction occurs exclusively through the specific-acid catalysis (i.e. no general-acid catalysis *by* H_2O was detected over a pH range of 1.0 to 4.0) (Allen, 1977). This would mean that the mechanistic interpretation of the observed rate constant (k_f or k_r) in the presence of the surfactant requires a careful scrutiny of the partition behavior of not only the prostaglandin but also H_3O^+ (Dougherty and Berg, 1974).

As shown in Table 4, at a constant concentration of prostaglandin (6.44×10^{-2} mg/ml; 1.75×10^{-7} M), both k_f and k_r decrease as the surfactant concentration increases. This is undoubtedly the consequence of increased F_m with the surfactant concentration (Table 1). In a 1.0% surfactant solution, in which over 95% of the total prostaglandin exists inside the micellar phase, k_f and k_r were reduced approximately 123- and 152-fold,

TABLE 4

THE FORWARD (k_f) AND THE REVERSE (k_r) RATE CONSTANTS FOR THE EPIMERIZATION OF 15(S)-15-METHYL $PGF_{2\alpha}$ AT pH 2.50 AND 25°C IN THE PRESENCE OF A CATIONIC SURFACTANT, MYRISTYL-γ-PICOLINIUM CHLORIDE

Percent surfactant	PG conc. (mg/ml)	k_f (10^6 s^{-1})	k_r (10^6 s^{-1})	K_{eq}
0	0.0644	208	385	1.85
0.05	0.0644	200	278	1.39
0.10	0.0644	59.5	83.3	1.40
0.20	0.0644	13.9	19.0	1.37
0.20	0.0322	10.2	18.8	1.84
0.20	0.0644	11.7	19.1	1.63
0.20	0.0966	14.7	21.9	1.50
0.20	0.2500	19.0	23.8	1.26
0.20	0.5152	19.5	26.9	1.39
0.20	0.9982	28.3	40.6	1.43
0.20	1.610	50.7	56.7	1.12
0.20	1.930	48.2	50.3	1.04
0.20	2.500	50.8	56.9	1.12
0.30	0.0644	6.67	10.4	1.56
0.40	0.0644	4.33	7.14	1.65
0.50	0.0644	3.05	4.30	1.41
1.00	0.0644	1.69	2.54	1.50

respectively. It is pointed out that the value of $F_m = 0.95$ merely indicates a *probability* of finding the prostaglandin molecules inside the micellar phase at a given time. In the previous section, two different binding sites in the micellar phase under discussion were postulated for the prostaglandin. In general, if the micellar phase provides z number of different binding sites for a given organic molecule, then Eqn. 1 should be modified as below:

$$k_{obs} = k_0\left(1 - \sum_{1=1}^{z} F_{m,i}\right) + \sum_{1=1}^{z} k_i F_{m,i} \tag{7}$$

where $k_1, k_2 \ldots k_z$ indicate the specific rate constant in a specific binding site. Thus the term $k_m F_m$ in Eqn. 1 should be considered an average of the linear combination shown in Eqn. 7.

The value of k_m can be estimated using Eqn. 8, which can be derived from Eqn. 1 (Fendler and Fendler, 1975);

$$\frac{1}{k_0 - k_{obs}} = \frac{1}{k_0 - k_m} + \frac{1}{k_0 - k_m}\frac{1}{K'}\frac{N}{(Q - CMC)} \tag{8}$$

where Q represents the total concentration of the surfactant and K′ is the apparent or overall association constant for the interaction between the prostaglandin molecules and

the micellar phase (K' should not be confused with K in Eqn. 5). Thus the plot of $1/(k_o + k_{obs})$ vs $1/(Q - CMC)$ should give a straight line. From the intercept one can estimate the magnitude of k_m. A double reciprocal plot is generally subject to an uneven error distribution unless a special care is taken in the experimental design. Thus the value of k_m obtained by Eqn. 8 should be considered as an approximate rather than an exact value. The data obtained at the surfactant concentrations greater than the CMC (Table 3) were plotted following Eqn. 8 and, as shown in Fig. 6, a k_m value of 6.2×10^{-7} s^{-1} can be obtained at 25°C.

The hydrocarbon *core* of the micellar phase formed by myristyl-γ-picolinium chloride should have a polarity comparable to that of an alkane, as in the case of Triton surfactant series (Podo et al., 1973). Based on the fact that 15-methyl $PGF_{2\alpha}$ is not soluble in *n*-hexane, one can postulate that the prostaglandin molecules may not penetrate deeply inside the micellar phase. It appears to be most likely that a short penetration of the prostaglandin into the palisade layer (close to the pyridine ring) provides the primary binding sites. Since the concentration of total prostaglandin was extremely low (1.75×10^{-7} M) for the experiments which resulted in Fig. 6, and since at such a low concentration of the drug only the primary binding sites are expected to be filled with the drug (See Tables 2 and 3, and Fig. 5), the value of k_m reported above is believed to reflect the epimerization rate at the palisade layer. Since the γ-picolinium cation is present in the vicinity of the primary binding site, the significant reduction of the rate constant observed appears to be primarily due to the electrostatic repulsion between the cationic head of the micelles and H_3O^+ rather than due to the polarity change.

Table 4 also shows the effects of the concentration of the total prostaglandin at a constant surfactant concentration (0.20%) upon the observed rate constants, k_f and k_r. As discussed in the previous section, a continuous addition of prostaglandin to a micellar solution is expected to force a secondary binding site to open up. The low affinity as well as the large number of binding sites indicates that the secondary binding sites are rather nonspecific. They can be a simple adsorption process onto the polar surface of the micelles. In this context, it is noteworthy that a Langmuir-type adsorption isotherm follows basically the same mathematical expression of the Scatchard equation (Eqn. 5).

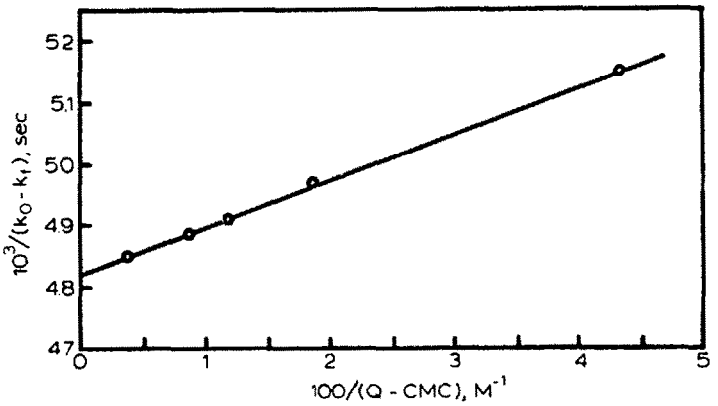


Fig. 6. Relationship between the observed epimerization constant (k_f) for 15(S)-15-methyl $PGF_{2\alpha}$ at pH 2.50 and 25°C and the surfactant concentration (Q) (see Eqn. 8 in the text).

Another possibility is the formation of mixed micelles. Various prostaglandins have been reported to be surface-active, especially when the total concentration begins to exceed 2×10^{-2} M (~7 mg/ml) (Roseman and Yalkowsky, 1973; Cho et al., 1977). Regardless of the exact location of the secondary binding site, the k_m value in this site must be greater than that of the primary bonding site to result in a steady increase in k_f and k_r as the prostaglandin concentration increases.

Although no attempts were made in the present report because of the large experimental errors, one should be able to resolve the observed k_m value into the specific k_m values for the primary and the secondary binding sites ($k_{m,1}$ and $k_{m,2}$ in Eqn. 7). First, one can estimate the ψ_m (and eventually F_m) values for the two types of binding sites at a given drug concentration directly from a graph such as Fig. 5. The variation of k_{obs} is then measured as a function of these ψ_m and F_m values, and Eqn. 7 solved simultaneously. To the present authors' knowledge, the effect of substrate concentration on the observed rate constant in the presence of a constant volume of the micellar phase has neither been a subject of numerous quantitative studies on the micellar catalysis nor attracted much attention among investigators. It is to be seen in the future if the above quantitative analysis is applicable in a complicated kinetic system.

During the course of the present study an interesting observation was made that the equilibrium constant for the reversible epimerization reaction (K_{eq} in Table 4 = k_r/k_f) is significantly away from unity; on average, K_{eq} = 1.44 favorable for 15(S)-epimer at pH 2.50 and 25°C. No proper explanations for this finding based on independent experimental data are provided at the present time. However, from a molecular study, it can be shown that the intramolecular hydrogen bonding between the C-15 hydroxyl group and the carboxyl group at C-1 is more plausible for 15(S)-epimer than 15(R)-epimer. This may also explain why 15(S)-15-methyl prostaglandins are generally eluted before 15(R)-epimers in an adsorption column chromatography (Morozowich, 1975).

ACKNOWLEDGEMENTS

The authors are thankful for the valuable discussion with Professor T. Sokoloski, School of Pharmacy, Ohio State University. The present report is an excerpt from M.A. Allen's B.A. Dissertation submitted to the Kalamazoo College, Kalamazoo, Michigan, 1977. His study was sponsored by The Upjohn Co., through the Senior Independent Project Program.

REFERENCES

Allen, M.A. The Effects of Surfactants on the Epimerization of 15(S)-15-Methyl $PGF_{2\alpha}$. B.A. Dissertation submitted to the Kalamazoo College, Kalamazoo, Mich., 1977.

Bender, M.L., Mechanisms of Homogeneous Catalysis from Protons to Proteins. Wiley, New York, 1971, pp. 368–373.

Bunton, C.A., Micellar catalysis and inhibition. In McCaldin, J.D. and Somorjai, G. (Eds.) Progress in Solid State Chemistry, Vol. 8, Pergamon Press, New York, 1973, pp. 239–281.

Cho, M.J., Kruezer, W.C. and Oesterling, T.O., Nucleophilic addition of bisulfite ion to prostaglandin E_2 and A_2: implication in aqueous stability. J. Pharm. Sci., 66 (1977) 149–154.

Cordes, E. (Ed.), Reaction Kinetics in Micelles. Plenum Press, New York, 1973.

Dougherty, S.J. and Berg, J.C., On the prediction of reaction rate in micellar solutions. J. Colloid Interface Sci., 49 (1974) 135–138.

Edsall, J.T. and Wyman, J., Biophysical Chemistry, Vol. I, Chap. 11, Academic Press, New York, 1958, pp. 591–662.

Fendler, J.H. and Fendler, E.J., Catalysis in Micellar and Macromolecular Systems, Academic Press, New York, 1975.

Foster, J.F., Plasma albumin. In Putnam, F.W. (Ed.) The Plasma Proteins, Vol. I, Academic Press, New York, 1960, pp. 179–239.

Frost, A.A. and Pearson, R.G., Kinetics and Mechanism. 2nd ed., Wiley, New York, 1961, p. 186.

Hart, H.E., Determination of equilibrium constants and maximum binding capacities in complex in vitro system. I. The mammillary system. Bull. Math. Biophys., 27 (1965) 87–90.

Higuchi, T. and Connors, K.A., Phase-solubility techniques. In Reilley, C.N. (Ed.) Advances in Analytical Chemistry and Instrumentation, Vol. 4, Wiley, New York, 1965, pp. 117–212.

Karim, S.M.M. (Ed.), Prostaglandins and Reproduction, MTP Press, Lancaster, 1975.

Krüger-Thimer, E., Dettli, L., Spring, P. and Diller, W., Mathematical problems in the calculation of protein binding of drugs with digital computers. In Estratto dal Vol. degli Att: del 3° Congr. Intern. di Medicina Cibernetica, Napoli, (1964, 1967), 1964, pp. 249–256.

Magerlein, B.J., DuCharme, D.W., Magee, W.E., Miller, W.L., Robert, A. and Weeks, J.R., Synthesis and biological properties of 16-alkylprostaglandins. Prostaglandins, 4 (1973) 143–145.

March, J., Advanced Organic Chemistry: Reactions, Mechanisms and Structure. McGraw-Hill, New York, 1968, pp. 125–142.

Mitchell, A.G., The hydrolysis of propyl benzoate in aqueous solutions of cetomacrogol. J. Pharm. Pharmacol., 15 (1963) 761–765.

Morozowich, W., Resolution of prostaglandin *p*-nitrophenacyl esters by liquid chromatography and conditions for rapid, quantitative *p*-nitrophenacylation. Prostaglandins, 10 (1975) 19–40.

Mukerjee, P. and Mysels, K.J., Critical Micelle Concentrations of Aqueous Surfactant Systems. Nat. Stand. Ref. Data Ser. 36, Nat. Bur. Stand. (U.S.), U.S. Government Printing Office, Washington, D.C., 1971.

Piszkiewicz, D., Positive cooperativity in micelle-catalyzed reactions. J. Am. Chem. Soc., 99 (1977) 1550–1557.

Podo, F., Ray, A. and Nemethy, G., Structure and hydration of nonionic detergent micelles. A high resolution nuclear magnetic study. J. Am. Chem. Soc., 95 (1973) 6164–6171.

Roseman, T.J. and Yalkowsky, S.H., Physicochemical properties of prostaglandin $F_{2\alpha}$ (thomethamine salt): solubility behavior, surface properties, and ionization constants. J. Pharm. Sci., 66 (1973) 1680–1685.

Rosenthal, H.D., A graphical method for the determination and presentation of binding parameters in a complex system. Anal. Biochem., 20 (1967) 525–529.

Rowe, E.L., Automated drop volume apparatus for surface tension measurement. J. Pharm. Sci., 61 (1972) 781–782.

Sandberg, A.A., Rosenthal, H.E., Schneider, S.L. and Slaunwhite, W.R. Jr., Theoretical and practical considerations in transcortin determination. In Nakao, T., Pincus, G. and Rait, J.F. (Eds.) Steroid Dynamics, Academic Press, New York, 1966, pp. 33–41.

Scatchard, G., Hughes, W.L., Jr., Gurd, F.R.N. and Wilcox, P.E., The interaction of protein with small molecules and ions. In Gurd, F.R.N. (Ed.) Chemical Specificity in Biological Interactions, Academic Press, New York, 1954, pp. 193–219.

Tanford, C., Physical Chemistry of Macromolecules. Wiley, New York, 1965, pp. 526–586.

Tanford, C., The Hydrophobic Effect: Formation of Micelles and Biological Membranes. Wiley, New York, 1973.

Weber, G., The binding of small molecules to proteins. In Pullman, B. and Weissbluth, M. (Eds.) Molecular Biophysics, Academic Press, New York, 1965, pp. 369–396.

Yankee, E.W., Axen, U. and Bundy, G.L., Total synthesis of 15-methylprostaglandins. J. Am. Chem. Soc., 96 (1974) 5865–5876.