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Uptake of Phenothiazine Tranquilizers by Lauromacrogol Micelles in Acidic Solution

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The uptake of the phenothiazine tranquilizers by lauromacrogol (PLE) micelles was studied in relation to ionic strength, pH and the chemical structure of the phenothiazines. The phenothiazine uptake could be described by a Langmuir type equation. The number of mol of chlorpromazine bound per mol of PLE at saturation, n, increased with increasing ionic strength, but the binding constant, K, changed in the opposite manner. The combined binding constant, nK, showed an almost constant value in the range of ionic strength up to 0.14 and then increased. There was no pH-dependency of the micellar uptake (pH 3.0—6.0) at constant ionic strength ($\mu=0.14$), indicating that only the cationic form of the phenothiazine was involved in the interaction. The value of nK was fairly well correlated with the critical micelle concentration of the phenothiazines. The results obtained suggest that the phenothiazine cations were oriented in PLE micelles so as to form mixed micelles.

Keywords——phenothiazines; lauromacrogol; micellar uptake; ionic strength effect; pH effect; chemical structure effect; mixed micelle

Many pharmacologically important drugs which are not primarily surfactants have been found to exhibit typical colloidal behavior in aqueous solution.[1] Among them are phenothiazine tranquilizers, which contain a hydrophobic phenothiazine ring and a hydrophilic ternary amino side chain (pK_a around 9[2]) in the molecule. Since the critical micelle concentrations (cmc) of these drugs are in the range of those of ionic surfactants,[3] the formation of mixed micelles with other ingredients such as surfactants which are often included in pharmaceutical dosage forms may be critical in relation to the biological effectiveness, even if the therapeutic concentrations are much lower than the cmc values of these drugs.[4]

The interaction of chlorpromazine, a typical phenothiazine tranquilizer, with nonionic surfactant micelles in water has been studied by dialysis,[5] ultraviolet absorption,[6] pH titration,[6,7] and proton magnetic resonance[7] measurements. These studies indicated that the nature of the interaction is hydrophobic. However, no quantitative study has been carried out to determine the degree of affinity of the phenothiazines for nonionic micelles.

When the cationic form of the phenothiazines and nonionic surfactants form mixed micelles, the colloidal properties of such micelles are likely to be influenced by ionic strength, as is well known in the case of usual ionic surfactant micelles.[8] Thus, in this study the effect of ionic strength on the uptake of chlorpromazine by lauromacrogol (PLE) micelles was examined in acidic solution and the results obtained were analyzed stoichiometrically. Further, structural effects were investigated by employing ten phenothiazines. The results cast some light on the mechanism involved.

Experimental

Materials——Chlorpromazine hydrochloride, promazine hydrochloride, promethazine hydrochloride, levomepromazine hydrochloride, perphenazine, and prochlorperazine methanesulfonate were supplied by Shionogi Pharmaceutical Co., Ltd. Trifluoperazine maleate and fluphenazine maleate were supplied by Yoshitomi Pharmaceutical Co., Ltd. Alimemazine tartrate was donated by Daiichi Pharmaceutical Co., Ltd. These phenothiazines were used as received.

Lauromacrogol (polyoxyethylene(23)lauryl ether) (PLE) was purified from commercially available Brij 35 as previously mentioned.[9] The critical micelle concentration (cmc) of PLE in water was reported to be 6.0—9.1 $\times 10^{-5}$ M at 25 °C.[10]

Cyclohexane used for the partitioning study was of spectrally pure grade. All buffer ingredients and sodium chloride were of reagent grade.

Equilibrium Dialysis——Buffer solutions used in the equilibrium dialysis and partitioning studies were 0.1 M acetate buffers which contained 0.1% sodium bisulfate to retard oxidation of phenothiazines. The ionic strengths of solutions were adjusted as required by adding sodium chloride. A bag (Visking cellulose tube (20/32)) was filled with 10 ml of 1×10^{-2} M surfactant solution and soaked in 40 ml of phenothiazine solution (2×10^{-4} to 6×10^{-4} M). The centrifuge tube containing the bag was shaken for a day at 30 °C under shielding from light. Samples from both sides of the bag were taken and assayed spectrophotometrically at around 260 nm for the phenothiazine. It was confirmed in preliminary experiments that the phenothiazines were satisfactorily stable under these experimental conditions.

Determination of Partition Coefficient——An aqueous solution (20 ml) of chlorpromazine (1×10^{-4} M) and 20 ml of cyclohexane were mixed and shaken for one hour at 30 °C, then allowed to stand for another hour. Samples of the aqueous phase were assayed. The apparent partition coefficient, P_{app}, was defined as the ratio of the equilibrium concentration in the organic phase to that in the aqueous phase.

Results and Discussion

In Fig. 1, the bound chlorpromazine concentration per mol of PLE, $(C_b)/(\text{PLE})$, is plotted against the unbound drug concentration, (C_f). It can be seen that $(C_b)/(\text{PLE})$ increases with increasing ionic strength at constant (C_f). It is of interest that at low ionic strengths such plots show a curvature, indicating a saturation phenomenon in the uptake of chlorpromazine by PLE micelles.

In the mathematical treatment of micellar interactions, either the partition or the solubilizate–micelle complex model has often been applied.[10,11] The former is based on the assumption that micellar species form a pseudophase and a solubilizate is partitioned between the micellar and aqueous phases, having the partition coefficient P_m. In the latter, the binding constant of the 1 : 1 complex, K_b, is simply defined. The respective constants are written as

$$P_m = \frac{(C_m)}{(C_a)} \cdot \frac{1}{\phi} \tag{1}$$

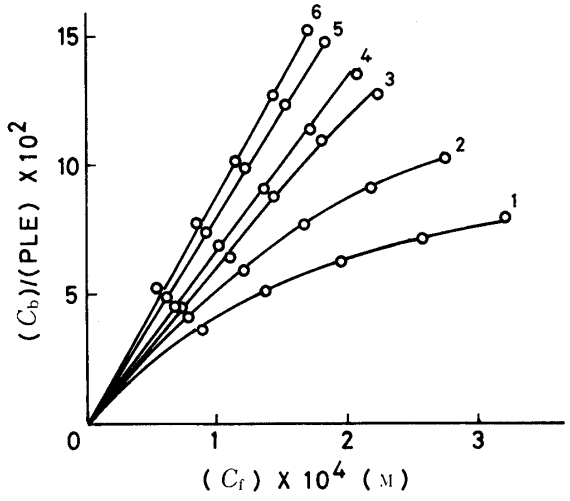


Fig. 1. Langmuir Plot for the Interaction of Chlorpromazine with PLE at pH 4.0 and 30 °C

The ionic strength of solution was 1, 0 (in distilled water); 2, 0.015; 3, 0.10; 4, 0.14; 5, 0.30; 6, 0.50.

$$K_b = \frac{(C_b)}{(C_f)(M)} \quad (2)$$

where (C_a) and (C_m) denote the concentrations of solubilizate in the aqueous and micellar phases expressed in terms of the volume of the system, respectively, ϕ is the volume fraction of micellar phase and (M) is the total concentration of the micelles under the condition $(M) \gg (C_b)$.

Equations (1) and (2) are essentially the same and hold only in the case of non-saturation of micellar interaction, *i.e.*, (C_b) (or (C_m)) increases linearly with increasing (C_f) (or (C_a)). Thus, in this study, the above equations could not be simply applied.

An attempt was made to apply a Langmuir type equation to the present results, *i.e.*,

$$\frac{(C_b)}{(\mathrm{PLE})} = \frac{nK(C_f)}{1+K(C_f)} \quad (3)$$

or its double reciprocal form

$$\frac{(\mathrm{PLE})}{(C_b)} = \frac{1}{n} + \frac{1}{nK(C_f)} \quad (4)$$

where K denotes the binding constant and n the number of mol of bound drug per mol of PLE at the hypothetical saturation of binding. When $(\mathrm{PLE})/(C_b)$ was plotted against $1/(C_f)$, a straight line was obtained in every case (plots not shown). The Langmuir equation has not often been applied in this way.[12]

From the estimated binding parameters (Table I), it can be seen that n increased with increasing ionic strength, but K decreased. At relatively high ionic strength ($\mu = 0.14$, 0.30 and 0.50) the plots according to Eq. (4) passed through the origin with good linearity and hence the parameters n and K could not be estimated separately.

Assuming that the aggregation number of PLE is 40 at 30 °C[10] and remains unchanged with respect to ionic strength, at the hypothetical saturation one PLE micelle could accommodate 5, 10 and 44 (n multiplied by 40) molecules of chlorpromazine in distilled water and in the solutions of ionic strength 0.015 and 0.10, respectively. This indicates that closer packing of chlorpromazine molecules onto the micelle occurs with increasing ionic strength, which is reminiscent of the well-known phenomenon that the aggregation number of ionic surfactants increases with increasing ionic strength because of the charge neutralization effect of counterions existing in the system.[8] On the other hand, closer packing means that a smaller region is available for the binding of each chlorpromazine molecule to the PLE micelle, resulting in decreasing K. Mutual compensation between increasing n and decreasing K means

TABLE I. Effect of Ionic Strength, μ, on the Binding of Chlorpromazine and PLE Micelles at pH 4.0 and 30 °C

μ	n	K (M^{-1})	nK (M^{-1})
0[a]	0.119	5.68×10^3	676
0.015	0.256	2.43×10^3	629
0.10	1.09	5.85×10^2	633
0.14	—[b]	—[b]	667
0.30	—[b]	—[b]	798
0.50	—[b]	—[b]	864

a) In distilled water.
b) Could not be estimated because the intercept of the plots according to Eq. (4) was almost zero within the range of experimental error.

that the combined binding constant, nK, which is considered to be a measure of the binding tendency of a compound,[12] may be maintained almost constant in the range of ionic strength 0—0.14. At higher ionic strength, nK showed a tendency to become larger. Although the reason is not clear, this may be due to gradual changes in the size and shape of the micelles caused by the added salt (sodium chloride).[13]

In the pH range where chlorpromazine exists mainly as a cationic form, a plot of the logarithm of the apparent partition coefficient between cyclohexane and aqueous solution, $\log P_{app}$, *versus* pH gave a straight line with a slope of unity, as shown in Fig. 2. The result indicates that only chlorpromazine base is partitioned into the organic phase and its partition coefficient is extremely large, *i.e.*, 2.0×10^5. The interior (the core) part of the PLE micelle is considered to be hydrophobic in nature, although its exterior (palisade layer) is hydrated and is rather hydrophilic.[14] It is, therefore, of importance to examine whether the base takes part in the binding to PLE micelles in acidic solution. Figure 2 also shows the pH-dependency of the nK value obtained at ionic strength 0.14. The result clearly indicates that under the present experimental conditions only the cationic form of the phenothiazine takes part in the interaction.

To achieve a further understanding of the mechanism involved, the binding of nine phenothiazines to PLE micelles was also studied at pH 4.0 and ionic strength 0.14. Under these conditions the plots according to Eq. (4) passed through the origin for all the penothiazines employed. The nK values obtained are listed in Table II together with the chemical structures of the phenothiazines.

From Table II, it appears that the phenothiazines with the same substituent at R_2, such as promethazine, alimemazine, promazine and perazine ($R_2=H$), or chlorpromazine, prochlorperazine and perphenazine ($R_2=Cl$), had comparable nK values. In contrast, for drugs with the same side chain at R_1 (3-(4-methyl-1-piperazinyl)propyl group), nK was dependent upon the substituent at R_2; the introduction of a hydrophobic substituent such as Cl and CF_3 led to a large nK. It is of interest that nK was fairly well correlated with the cmc values of the phenothiazines, as shown in Fig. 3. The lower the cmc of the phenothiazine, the greater the binding to PLE micelles. This indicates that an amphiphilic monomer with lower cmc is more

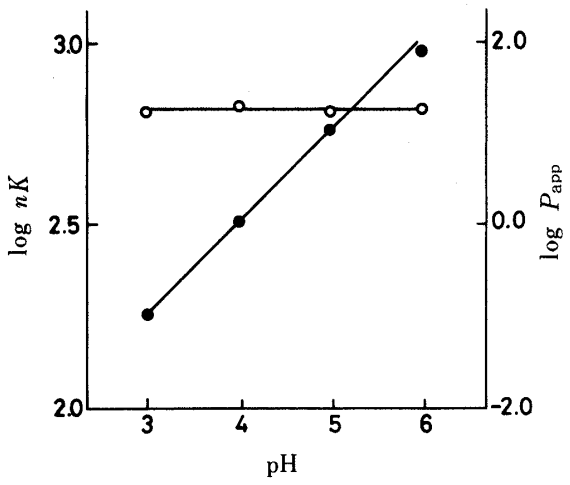


Fig. 2. pH-Dependencies of the Combined Binding Constant (nK), (○), and Apparent Partition Coefficient (P_{app}), (●), of Chlorpromazine

The experiment was carried out in 0.1 M acetate buffer with total ionic strength 0.14 at 30 °C.

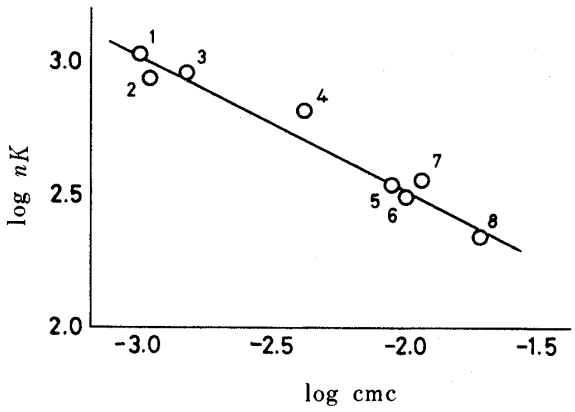
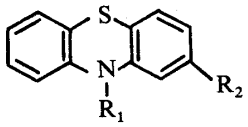


Fig. 3. Relationship between the Combined Binding Constants (nK) of Phenothiazines and Their Critical Micelle Concentrations (cmc)

The cmc values of phenothiazines were taken from Ref. 3. 1, trifluoperazine; 2, perphenazine; 3, fluphenazine; 4, chlorpromazine; 5, levomepromazine; 6, alimemazine; 7, promazine; 8, promethazine.

TABLE II. Chemical Structures of Phenothiazines Used and the Combined Binding Constant, nK, at pH 4.0 (μ=0.14) and 30 °C



Compound	R_1	R_2	nK (M^{-1})
Promethazine	$CH_2CH(CH_3)N(CH_3)_2$	H	225
Alimemazine	$CH_2CH(CH_3)\ CH_2N(CH_3)_2$	H	318
Levomepromazine	$CH_2CH(CH_3)\ CH_2N(CH_3)_2$	OCH_3	347
Promazine	$CH_2CH_2CH_2N(CH_3)_2$	H	363
Chlorpromazine	$CH_2CH_2CH_2N(CH_3)_2$	Cl	667
Perazine	$CH_2CH_2CH_2N\ NCH_3$	H	372
Prochlorperazine	$CH_2CH_2CH_2N\ NCH_3$	Cl	898
Trifluoperazine	$CH_2CH_2CH_2N\ NCH_3$	CF_3	1080
Perphenazine	$CH_2CH_2CH_2N\ NCH_2CH_2OH$	Cl	877
Fluphenazine	$CH_2CH_2CH_2N\ NCH_2CH_2OH$	CF_3	916

surface-active, having a greater affinity to PLE micelles. It is, therefore, very likely that the cationic form of the phenothiazines is oriented in such a way that the hydrophobic phenothiazine ring is in the hydrophobic part of the PLE micelle and the protonated side chain is at the micelle surface, contributing to the formation of a mixed micelle.

In a study of the solubilization of chlorhexidine diacetate and dichloride by deca-oxyethylene oleic ether (Brij 96), Wesoluch *et al.* concluded that the cationic form of chlorhexidine is solubilized into the micellar interior as the ion-pair.[15] Although the ion-pair mechanism is another possibility here, it seems unlikely to apply to phenothiazines, since, if this were the case, the uptake of phenothiazine should be linear with respect to free (unbound) drug concentration instead of showing a saturation phenomenon (Fig. 1).

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