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Micellization of an Aqueous Solution of Piperidolate Hydrochloride in the Presence of Acetylcholine Chloride

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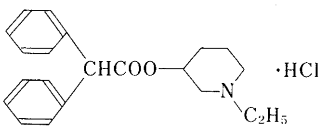
The surface tension of an aqueous solution of piperidolate hydrochloride (PIH), which is an anti-cholinergic drug, was measured with a Du Nöuy tensiometer at 25 °C in the absence and presence of acetylcholine chloride (Ach·Cl), and the effect of Ach·Cl on the critical micelle concentration (cmc) of PIH was investigated by comparing it with the effects of sodium chloride (NaCl) and calcium chloride ($CaCl_2$). Furthermore, the effect of Ach·Cl on the cmc of PIH was discussed by comparing it with the effect of Ach·Cl on the cmc of dodecyltrimethylammonium chloride (DTAC). The value of cmc of PIH decreased with an increasing concentration of Ach·Cl. The order of the effect on the decrease in the cmc of PIH was $CaCl_2$ > NaCl > Ach·Cl. The effect of Ach·Cl on the decrease in the cmc of PIH was smaller than that on the decrease in the cmc of DTAC.

Keywords piperidolate hydrochloride; critical micelle concentration; acetylcholine chloride; surface tension; micelle

Piperidolate hydrochloride (PIH) is an anti-cholinergic drug. It is important to study the surface properties of PIH in connection with its pharmacological effects at the surface of a biological membrane. The aggregation of PIH in water at 30 °C has been studied by the light scattering method.[1] However, the surface tension of an aqueous solution of PIH has not been measured, and the critical micelle concentration (cmc) in the presence of additives has not yet been obtained. Acetylcholine chloride (Ach·Cl) is a cholinergic drug and is a pharmacological antagonist to PIH. In addition, Ach·Cl is an organic electrolyte, which has both a hydrophobic and a polar moiety. It is interesting to study the effect of Ach·Cl on the surface active properties of PIH from the viewpoint not only of surface chemistry but also pharmacology. Therefore, we measured the surface tension of aqueous solutions of PIH in the presence of Ach·Cl or inorganic electrolytes, and then investigated the effect of additives on the cmc of PIH. Furthermore, the effect of Ach·Cl on the cmc of PIH was discussed by comparing with the effect of Ach·Cl on the cmc of dodecyltrimethylammonium chloride (DTAC).[2]



PIH

$CH_3COO(CH_2)_2N^+(CH_3)_3 \cdot Cl^-$

Ach·Cl

Experimental

Materials PIH and Ach·Cl, purchased from Wako Pure Chemical Ind., Ltd., were of a guaranteed reagent grade (the purities were 99.0 and 99.5%, respectively) and were used without further purification. The chemical structures of PIH and Ach·Cl are shown in Chart 1. Sodium chloride (NaCl) and calcium chloride ($CaCl_2$) were of analytical reagent grade. All solutes were dissolved in deionized and redistilled water.

Measurement of Surface Tension The surface tension was measured with a Du Nöuy tensiometer. The platinum ring with a diameter of 19 mm was heated by an oxidizing flame before use. The thermostat temperature was maintained at 25±0.1 °C. For the calculation of the surface tension of aqueous solutions, the value of 71.96 mN m^{-1} was used as the surface tension of pure water at 25 °C. The experimental determination of the surface tension was precise to ±0.1 mN m^{-1}.

The aqueous solution of Ach.Cl is stable at 25 °C under neutral–acidic conditions, although the hydrolysis of Ach.Cl is significant above pH 9: the percentage of hydrolyzed Ach·Cl at pH 7.6 and 25 °C for 1 h was about 0.3%, although the percentage of hydrolyzed Ach·Cl at pH 9.0 and 25 °C for 1 h was about 8%.[3] In this study, the pH of the aqueous solution of PIH was below 7 and the time required for the measurement of surface tension was about 1 h. Therefore, the hydrolysis of Ach·Cl is negligible in this study.

Results and Discussion

Surface Tension of Aqueous Solution of PIH The change in surface tension (γ) of PIH in water as a function of the logarithm of concentration was shown in Fig. 1, where C is the concentration of PIH. In the γ *vs.* log C curve for the PIH–water system, an inflection point was found, but the inflection was not sharp. This is considered to be due to the fact[4] that surfactants with aromatic hydrophobic groups are generally thought to associate by a process in which aggregate growth occurs by the continuous stepwise addition of monomers. The blunt inflection was not caused by an impurity because there was no minimum in the curve and PIH has high purity. As is shown in Fig. 1, the slope of the curve becomes gentle at 6—7 × 10^{-2} M of PIH, and the value of γ is nearly constant at 9—15 × 10^{-2} M. (The value of γ at

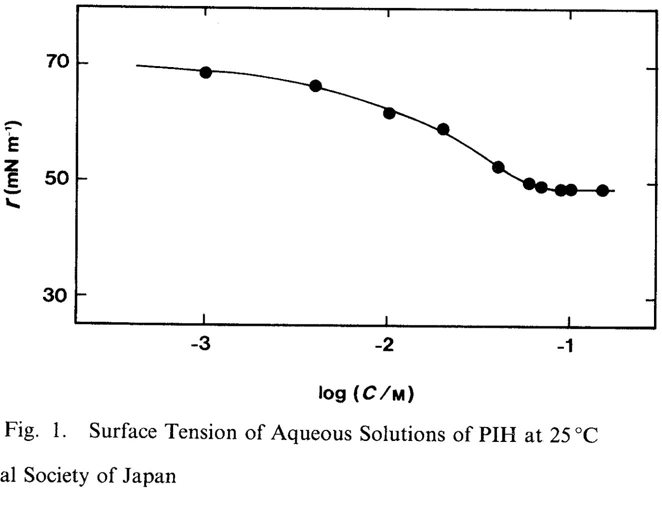


Fig. 1. Surface Tension of Aqueous Solutions of PIH at 25 °C

concentrations above 15×10^{-2} M could not be measured because of the limited solubility of PIH.) The value of cmc was estimated as the point of intersection of the extension of the gentle curve and horizontal line: the cmc of PIH in water was obtained as 8.1×10^{-2} M. The cmc of PIH in water at 30 °C is reported to be 8.2×10^{-2} M.[1] So, the value obtained in this study is considered to be adequate.

Next, the plots of γ *vs.* log C in the presence of additives (10 mM of $CaCl_2$ and 20 mM of Ach·Cl) are shown in Fig. 2. The inflection points are clearly found in the presence of salts as compared with that in the absence of salt. These phenomena are consistent with the fact[5] that a tetracaine hydrochloride–water system shows no distinct inflection point, but a tetracaine hydrochloride–0.4 M NaCl system shows an abrupt inflection point.

Effect of Ach·Cl on the cmc of PIH The relationship between the cmc of PIH and the concentration of Ach·Cl is shown as closed circles in Fig. 3, where C_{additive} is the concentration of the additive (Ach·Cl, NaCl or $CaCl_2$). The cmc of PIH decreased with increasing concentrations of Ach·Cl, indicating that Ach·Cl acts as an additive salt although Ach·Cl is a pharmacological antagonist to PIH. Next, the effect of Ach·Cl on the cmc was compared with that of inorganic salts. The relationships between the cmc of PIH and the concentration of NaCl or $CaCl_2$ are shown as open circles and semi-open circles, respectively in Fig. 3. As can be seen in Fig. 3, the order of the effect on the decrease in the cmc of PIH was $CaCl_2 > NaCl >$ Ach·Cl. The moiety of $-C_5H_9N-C_2H_5 \cdot HCl$ in the PIH molecule can be changed into $-C_5H_9N^+H-C_2H_5 \cdot Cl^-$. Thus, the PIH micelle is considered to be positively charged. We will measure the surface potential of the PIH micelle. NaCl and $CaCl_2$ decreased the cmc of PIH by a shielding effect of the electrostatic repulsion at the micelle surface. One molecule of $CaCl_2$ produces two chloride ions by dissociation, while one molecule of NaCl produces one chloride ion. So, the shielding effect of $CaCl_2$ is larger than that of NaCl. Ach.Cl also decreases the cmc of PIH by a shielding effect of the chloride ion, while penetration of the Ach^+ into the PIH micelle prevents the PIH molecule from associating by creating an electrostatic repulsive force between the head groups. It has been found[6] that Ach·Cl enters not only in anionic micelles but also in cationic micelles. Therefore, the effect of Ach·Cl on the decrease in the cmc of PIH is smaller than that of NaCl.

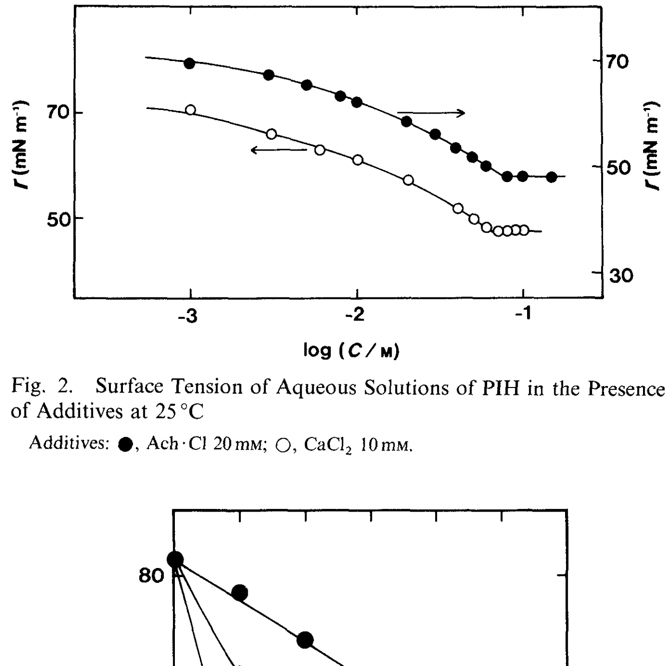


Fig. 2. Surface Tension of Aqueous Solutions of PIH in the Presence of Additives at 25 °C

Additives: ●, Ach·Cl 20 mM; ○, $CaCl_2$ 10 mM.

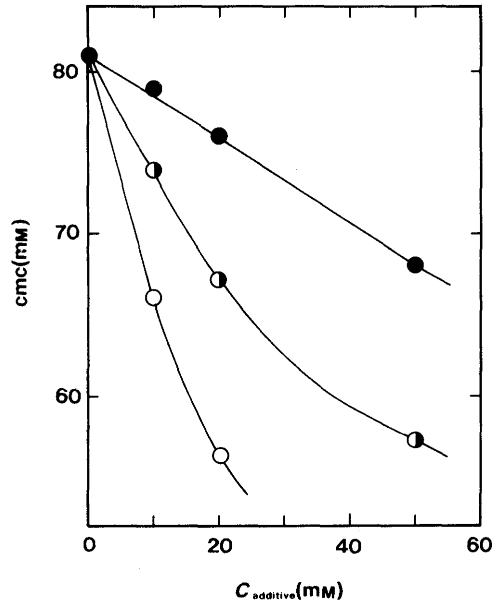


Fig. 3. Effect of Additives on the cmc of PIH at 25 °C

Additives: ●, Ach·Cl; ◑, NaCl; ○, $CaCl_2$.

Next, the effect of additives on the cmc of PIH was compared with that of additives on the cmc of DTAC as a cationic surfactant. The values of the cmc of PIH and DTAC[2] in the presence of 10 mM of NaCl and Ach·Cl are summarized in Table I. The effect of NaCl on the decrease in the cmc of PIH is smaller than that of NaCl on the decrease in the cmc of DTAC. ((81—74) × 100/81 ≒ 8.6 for PIH, while (16—12) × 100/16 = 25 for DTAC). This is considered to be caused by the smaller aggregation number of PIH micelles than that of DTAC micelles, so that the shielding effect by a counter ion cannot be significantly noted. The micellar aggregation number of PIH in water is 12,[1] while that of DTAC in water is 50.[7] In addition, the effect of Ach·Cl on the decrease in the cmc of PIH is also smaller than that of Ach·Cl on the decrease in the cmc of DTAC. However, the difference in the effectiveness of Ach·Cl between PIH and DTAC is not as small as the difference in the effectiveness of NaCl between PIH and DTAC. This is considered to be due to the fact that the amount of Ach^+ which penetrates into the PIH micelle is smaller than that entering the DTAC micelle, leading to the smaller electrostatic repulsive force between PIH and Ach^+. This may be caused by the plate-like structure of the PIH micelle.

The values of surface tension at the cmc (γ_{cmc}) of PIH and DTAC[2] are also listed in Table I. The value of γ_{cmc}

TABLE I. Effect of Additives on the cmc and γ_{cmc} of PIH and DTAC at 25 °C

Additives	cmc (mM)		γ_{cmc} ($mN\,m^{-1}$)	
	PIH	DTAC[2]	PIH	DTAC[2]
Absence	81	16	49	40
Ach·Cl 10 mM	79	15	48.5	38.5
NaCl 10 mM	74	12	47.5	36

of PIH is larger than that of DTAC, which also indicates the weaker surface activity of PIH compared to DTAC. The effect of Ach·Cl and NaCl on the γ_{cmc} of PIH was similar to that on the cmc of PIH. As a result, the surface activity of PIH was enhanced in the presence of Ach·Cl, suggesting stronger pharmacological effects of PIH at the surface of a biological membrane. As for medication involving PIH, however, 150 mg are given to patients in a divided dose three times per day. It is considered that the dosage of PIH is too small for any significant surface activity of PIH to appear.

In conclusion, the order of the effect on the decrease in the cmc of PIH was $CaCl_2 > NaCl > Ach \cdot Cl$. This phenomenon is explained by the electrostatic shielding effect and by the distribution of Ach^+ in the PIH micelle.

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