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COMMUNICATION

# A Temperature Study on Critical Micellization Concentration of the Novel Platelet-Activating Factor Receptor Antagonist E5880 in Water by Electric Conductivity Measurements

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**ABSTRACT**

*To clarify the behavior of the novel platelet-activating factor (PAF) receptor antagonist E5880 in aqueous solution, electric conductivity was measured at different temperatures (every 5°C), ranging from 15°C to 50°C. Critical micellization concentration (CMC) of E5880 was dependent on the temperature; at 30°C, the CMC value was smallest (0.143 mM). Below that temperature, the enthalpy for formation of the micelle ($\Delta H_m^0$) was positive, and the formation of micelles was endothermic; above that temperature, $\Delta H_m^0$ was negative, and the formation of micelles was exothermic.*

## INTRODUCTION

Platelet-activating factor (PAF) exhibits a variety of biological activities, including activation of platelets (1), neutrophils (2), bronchoconstriction (3), hypermeability in peripheral veins (4), hypotension (5), and cardiac dysfunction (6). Because these biological activities of PAF are extremely potent, it is generally accepted that PAF is a mediator of inflammation (7) and plays important roles in the pathology of thrombosis, asthma, or hypotension in shock (8–10). Consequently, it is expected that specific PAF receptor antagonists may be beneficial for the treatment of these diseases, and many efforts to develop potent and specific PAF antagonists have been made.

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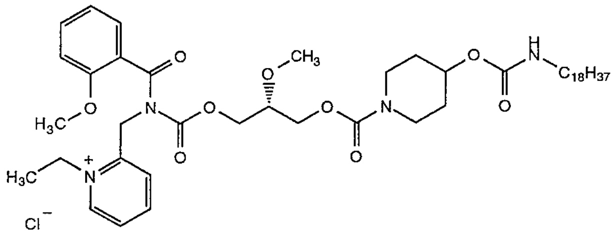


**Figure 1.** Chemical structure of the platelet-activating factor (PAF) antagonist E5880.

E5880, a newly synthesized PAF antagonist (Fig. 1), is more potent in PAF receptor binding than PAF (11). This compound is amphiphilic, and it is expected to form the aggregates in aqueous media. For the treatment of the above diseases, an injectable formulation would be extremely useful. To develop the injectable formulation, the clarification of the characteristics of the physicochemical properties for E5880 micelle is important.

In this study, the critical micellization concentration (CMC) of E5880 and degree of counterion binding β were determined by electric conductivity measurements at different temperatures. The changes in the Gibbs energy $\Delta G^0_m$ enthalpy, $\Delta H^0_m$, and entropy $\Delta S^0_m$ on micelle formation as a function of temperature were evaluated taking β values into calculation.

## EXPERIMENTAL

### Materials

E5880 was obtained from Eisai Chemical Company, Limited (Ibaraki, Japan). Sodium chloride was purchased from Tokyo Kasei Company, Limited (Tokyo, Japan).

### Determination of Critical Micellization Concentration and the Degree of Counterion Binding β

All of the CMC data were obtained from electric conductivity measurements using a TOA electric conductivity meter, model CM-40S (TOA Co., Ltd., Tokyo, Japan). A CMC was determined from conventional plots of specific conductivity $K$ versus analytical concentration $C$ at the temperatures ranging from 15°C to 50°C (at every 5°C). The temperature dependence of CMC for E5880 at different concentrations of added NaCl was determined.

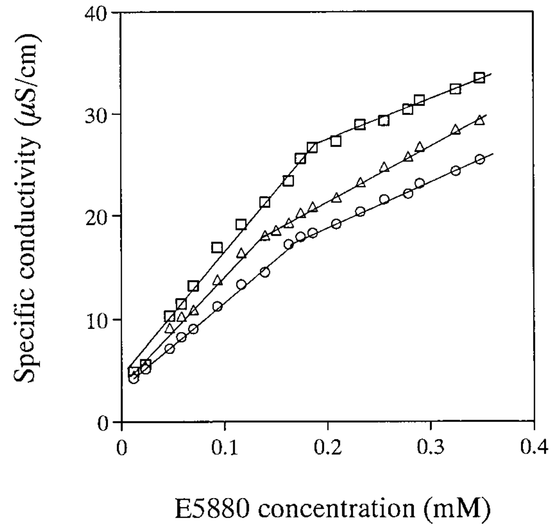


**Figure 2.** Specific conductivity $K$ versus concentration of E5880 $C$ in pure water: ○, 15°C; △, 30°C; □, 45°C.

## RESULTS AND DISCUSSION

### Determination of the Temperature Dependence of the Critical Micellization Concentration of E5880

The CMC values were estimated from the break points in the plots of specific conductivity $K$ versus the concentration of E5880 (Fig. 2) and are presented in Table 1.

Figure 3 shows the relationship between temperature and CMC as a function of added salt concentration. All the curves have a minimum around 30°C, as observed for different ionic surfactants (12–15). From Fig. 2, the added salt effect of CMC it is clearly seen, although the

***Table 1***

*Critical Micelle Concentration and Degree of Counterion Binding β at Discrete Temperatures*

| Temperature (°C) | CMC (mM) | β |
|---|---|---|
| 15 | 0.160 | 0.718 |
| 20 | 0.152 | 0.720 |
| 25 | 0.148 | 0.722 |
| 30 | 0.143 | 0.725 |
| 35 | 0.150 | 0.721 |
| 40 | 0.164 | 0.719 |
| 45 | 0.173 | 0.717 |
| 50 | 0.178 | 0.715 |

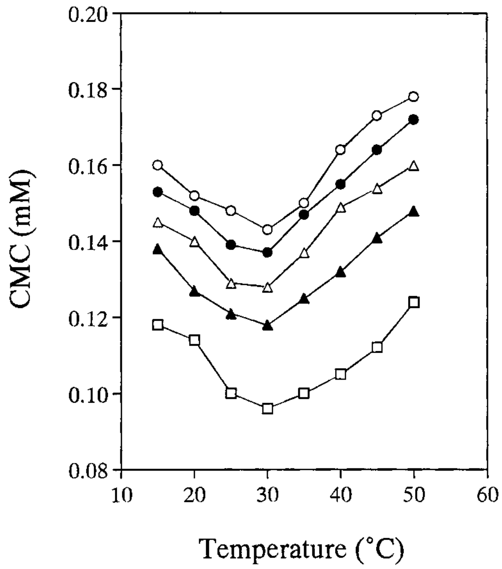


**Figure 3.** The temperature dependence of CMC for E5880 at different concentrations of added NaCl: ○, absence of NaCl; ●, 0.3 mM NaCl; △, 0.5 mM NaCl; ▲, 0.7 mM NaCl; □, 1.0 mM NaCl.

concentration of the added salt is restricted to a narrow range below 1 mM.

## Estimation of the Degree of Counterion Binding β

The curves are used for estimating the degree of counterion binding to micelles β at each temperature; the β is given from the following relations:

$$\Delta G^0_m = RT \ln X_{cmc}(X_{cmc} + X_a)^{\beta} \quad (1)$$

or

$$\ln X_{cmc} = \Delta G^0_m/RT - \beta \ln(X_{cmc} + X_a) \quad (2)$$

where $\Delta G^0_m$ is the standard Gibbs energy change on micelle formation, $X_{cmc}$ and $X_a$ are concentrations in mole fraction of the E5880 at CMC and of the added salt in aqueous media, respectively, and $RT$ is the product of the molar gas constant temperature in kelvin. From Eq. 2, the CMC as a function of added salt concentration is more concisely expressed as ln CMC = const $(T,P) - \beta \ln C_g$.

This equation indicates that the β corresponds to the slope of the curve in the plot of logarithmic CMC against logarithmic counterion (gegenion) concentration (i.e., $C_g$ = CMC + concentration of added salt); this is the so-called Corrin-Harkins plot. These plots showed good linearity, with a regression of more than 0.98 at each temperature. Being plotted against temperature, the β increases a little and then decreases beyond a maximum with increased temperature (not shown here). The β values thus determined from the slopes and the CMC data (in millimolality) of the systems in pure water at the respective temperatures are shown in Table 1.

## Thermodynamic Analysis for the Formation of E5880 Micelles

On the basis of the data in Table 1, let us try a thermodynamic analysis and discussion. Applying the following

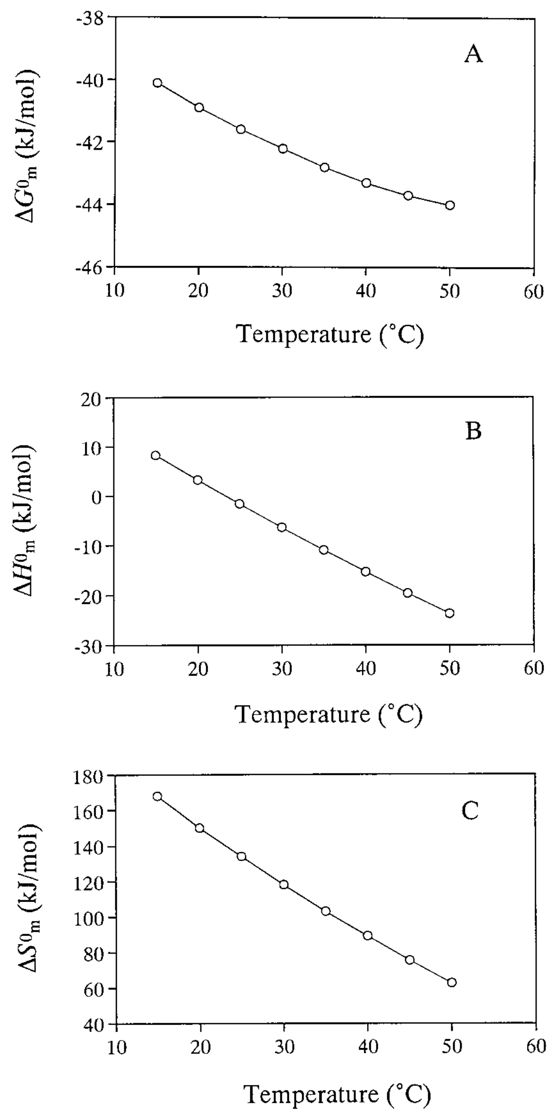


**Figure 4.** Thermodynamic parameters on E5880 micelle formation as a function of temperature: (A) Gibbs energy change $\Delta G^0_m$; (B) the enthalpy change $\Delta H^0_m$; (C) the entropy change $\Delta S^0_m$.

equation derived from Eq. 1 when no salt is added ($X_a = 0$), the standard Gibbs energy change can be calculated using the basic data in Table 1:

$$\Delta G^0_m = (1 + \beta)RT \ln X_{cmc} \qquad (3)$$

Here, it is well known that, strictly described, this equation is derived from the charged phase-separation model (15).

The well-known van't Hoff plot was applied for evaluating the enthalpy change on micelle formation $\Delta H^0_m$ from the Gibbs energy change; the obtained curve is shown in Fig. 4A. Here, the product of the slope with the gas constant corresponds to the enthalpy change as follows:

$$[\partial(\Delta G^0_m/RT)/\partial(1/T)]_p = \Delta H^0_m/R \qquad (4)$$

Further, using the estimated $\Delta H^0_m$ value, the entropy change $\Delta S^0_m$ can be calculated from the relation

$$\Delta S^0_m = (\Delta H^0_m - \Delta G^0_m)/T$$

In Figs. 4B and 4C, $\Delta H^0_m$ and the entropy term $\Delta S^0_m$ are plotted against the temperature in kelvin, respectively. Figure 4 shows that the enthalpy term changes from positive (endothermic) to negative (exothermic) at the temperature corresponding to the minimum of the CMC-temperature curve; on the other hand, the entropy term decreases monotonously with temperature. These results indicate that, below 30°C, $\Delta H^0_m$ is positive, and the formation of micelles is endothermic; above 30°C, $\Delta H^0_m$ is negative, and the formation of micelles is exothermic.

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