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Author	藤野, 美弘(Fujino, Yoshihiro) 藤江, 忠雄(Fujie, Tadao) 横山, 祥子(Yokoyama, Shoko)
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Micellization of Dibucaine Hydrochloride (DC) in Water by Measurement of the Fluorescence of DC

Yoshihiro FUJINO, Tadao FUJIE, and Shoko YOKOYAMA

藤野 美弘, 藤江 忠雄, 横山 祥子

The micellization of dibucaine hydrochloride (DC) in water at 25℃ was investigated by measuring the fluorescence spectrum of DC. Furthermore, the surface tension of an aqueous solution of DC was measured with a Du Nöuy tensiometer at 25℃. The critical micelle concentration of DC in water at 25℃ was estimated as $7 \sim 8 \times 10^{-2}$ M by the fluorescence method, and this value is coincided with the value of 7.4×10^{-2} M by the surface tension method.

Key words dibucaine hydrochloride; micelle; fluorescence spectrum; critical micelle concentration; surface tension

Dibucaine hydrochloride (DC) is a local anesthetic drug. It is important to study the surface activity of DC in connection with its pharmacological effects at the surface of a biological membrane since the parallelism between the surface activity and the pharmacological potency was proposed by Levy[1]. The aggregation of DC in water has been studied by the light scattering method[2] and the drop volume method[3].

On the other hand, ammonium 8-anilino-1-naphthalene-sulfonate (ANS) as a fluorescent probe has been used for the measurements of critical micelle concentration (cmc)[4], critical reversed micelle concentration[5] and the surface potential of the micelles[6]. This is based on the fact that ANS adsorbed on the micelle surface fluoresces strongly although the fluorescence of free ANS is very weak. Recently, the study on the pK_a of DC by measuring the fluorescence of DC has been reported[7]. The micellization of non-fluorescent compound can be determined by the ANS method, but the micellization of fluorescent compound can not be accurately determind by the ANS method because fluorescence intensity increases with increasing concentration of fluorescent compound. By the way, it is well known that the physicochemical properties of surfactant are largely changed at the cmc. So the maximum wavelength of emission spectrum and the quantum yield of fluorescent compound may be changed near the cmc. The fluorescence of DC has been reported as a function of pH[7]. However, the fluorescence of DC has not yet been determined by varying the concentration of DC.

From these points of view, we measured the fluorescence spectrum of aqueous solution of DC at various concentrations and estimated the cmc of DC. Furthermore, the cmc of DC obtained from the fluorescence method was compared with that obtained from the surface tension method.

Experimental

Materials Dibucaine hydrochloride (DC) purchased from Wako Pure Chemical Industries, Ltd. was recrystallized twice from methanol. The molecular structure of DC is shown in **Chart 1**. Deionized and twice-distilled water was used throughout this study.

Measurement of Fluorescence Spectrum DC was dissloved in water. The fluorescence spectra were measured with a Hitachi F-4000 spectrofluorometer as previously described[6]. The temperature was

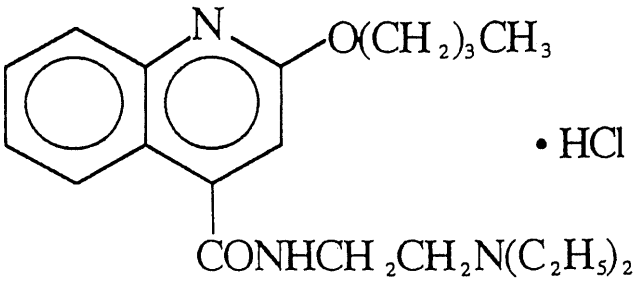

Chart 1 Molecular Structure of Dibucaine Hydrochloride (DC)

maintained at 25±0.2℃ during the fluorescence measurement by circulating water through the cuvette holders. The fluorescence spectra were determined after temperature of the sample solution had reached the measurement temperature. The wavelength of excitation was 373 nm. Louro *et al.*[7] reported that the wavelength of excitation (λ_{ex}) for the aqueous solution of DC was 328 nm. However, we obtained 373 nm of λ_{ex} when we searched the suitable λ_{ex} at a fixed wavelength of emission. Thus, we measured the fluorescence spectra of aqueous solutions of DC at excitation wavelength 373 nm.

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℃. 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℃. The experimental determination of the surface tension was precise to ±0.1 mN m^{-1}.

Results and Discussion

Maximum Wavelength of Emission Changes in the maximum wavelength of the emission spectrum (λ_{max}) of DC excited at 373 nm by varying the concentration of DC from 0 to 0.2 M are shown in **Fig. 1**, where C is the concentration of DC. DC in water had a λ_{max} at 418 nm, and λ_{max} was constant at concentrations up to 4×10^{-2} M. At 6×10^{-2} M λ_{max} shifted largely to a longer wavelength. At 8×10^{-2} M λ_{max} further shifted to a longer wavelength, and the value of λ_{max} remained constant at concentrations above 8×10^{-2} M. These phenomena are considered to be caused by that the premicellar associations begins to be formed at about 6×10^{-2} M and that the micelles are formed at concentrations above 8×10^{-2} M. In general, the changes in fluorescence followed by a premicellar association are larger as compared with the other physicochemical parameters. In addition, surfactants with aromatic hydrophobic groups associate by a process[8] in which aggregate growth occurs by the continuous stepwise addition of monomers. The cmc of DC in water at 25℃ is estimated as $7\sim8\times10^{-2}$ M. The cmc of DC in water is reported as $6.0\sim6.6\times10^{-2}$ M at 30℃ by the light scattering method[2] and 7.87×10^{-2} M at 25℃ by the drop volume technique. So the value of cmc of DC obtained by the fluorescence method is considered to by adequate.

Surface Tension of Aqueous Solution of DC The surface tension (γ) of DC in water as a function of log C was presented in **Fig. 2**. As can be seen in **Fig. 2**, γ decreased with increasing concentration of DC and became nearly constant at concentrations of DC above 8×10^{-2} M. The inflection point in the γ ***vs.*** log C curve is not sharp. This phenomenon is satisfied with the results shown in **Fig. 1**. This is considered to be based on the association mechanism of DC in which aggregate growth occurs by the continuous stepwise addition. The value of cmc in the γ ***vs.*** log C curve was estimated as the point of intersection of the extension of the gentle curve and horizontal line: the cmc of DC in water was obtained as 7.4×10^{-2} M. This value is coincided with the value obtained by the fluorescence method.

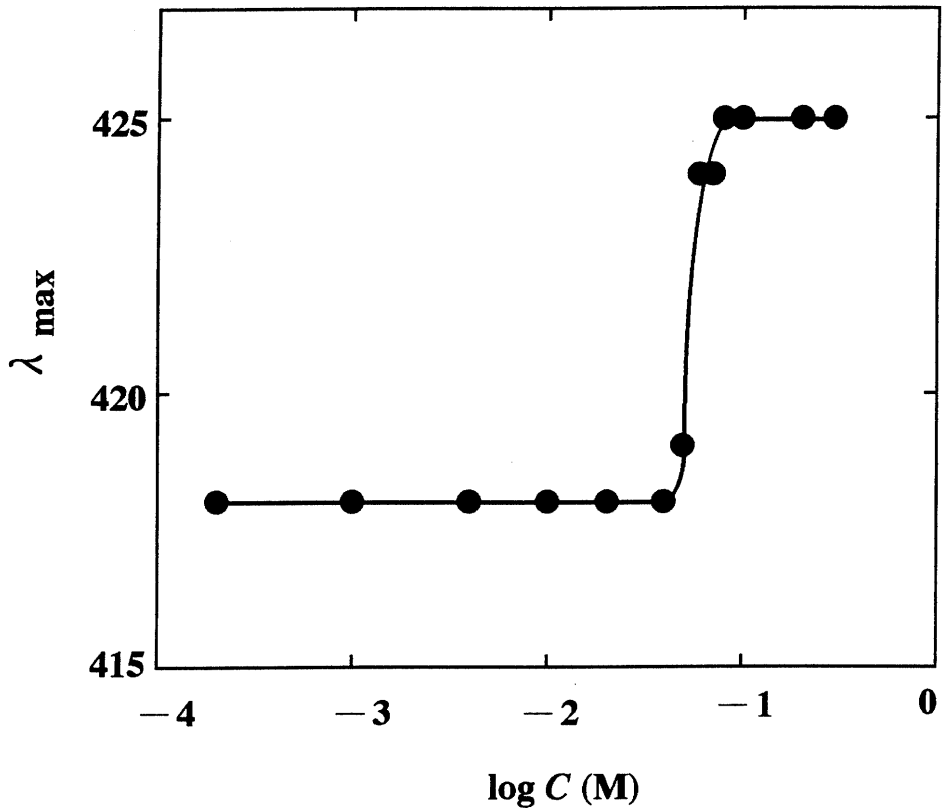

Fig. 1 Variation of λ_{max} of DC with Increasing Concentration of DC

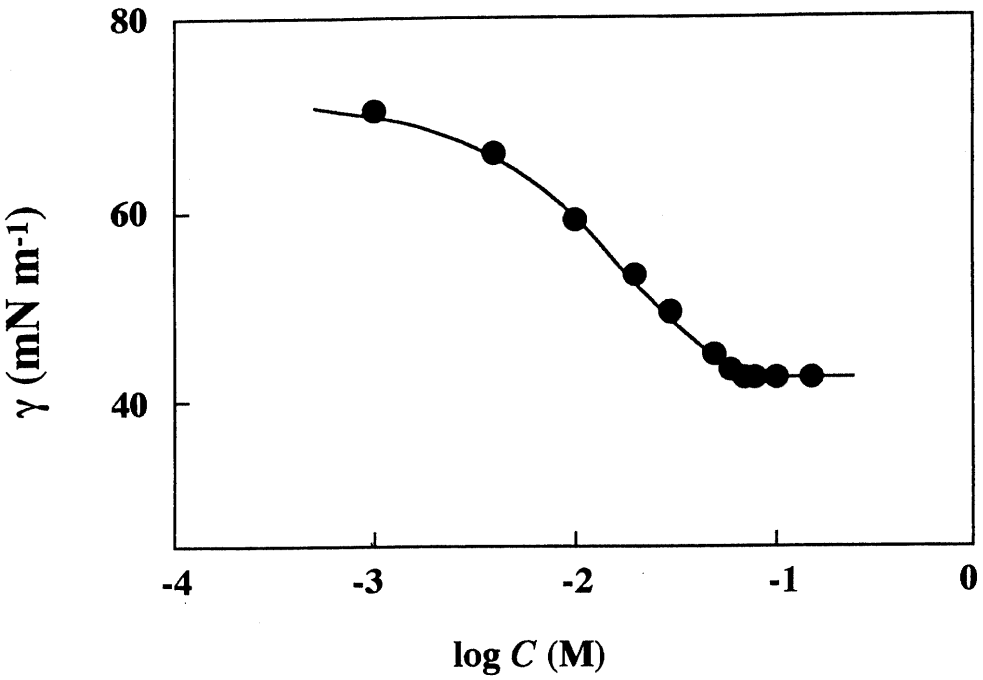

Fig. 2 Surface Tension of Aqueous Solutions of DC at 25℃

In conclusion, the micellization of DC in water can be determined by measuring the self-fluorescence of DC. The cmc of DC in water at 25℃ was obtained as $7 \sim 8 \times 10^{-2}$ M by the fluorescence method, and this value was coincided with the cmc value of 7.4×10^{-2} M obtained by the surface tension method.

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