Chem. Pharm. Bull. **45**(12) 2104—2106 (1997) Vol. 45, No. 12

Surface Potential of Mixed Micelles Composed of Dibucaine Hydrochloride and Heptaethylene Glycol Dodecyl Ether

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Received July 11, 1997; accepted September 13, 1997

The surface potential ($\Delta\psi$) of the mixed micelles composed of dibucaine hydrochloride (DC) and nonionic heptaethylene glycol dodecyl ether (HED) has been estimated by measuring the fluorescence intensity of ammonium 8-anilino-1-naphthalenesulfonate (ANS) as a fluorescent probe. The value of $+\Delta\psi$ increases with an increasing mole fraction of DC (X_{DC}) and decreases with increasing ionic strength (I). These changes are due to the effects of the positive charge of DC and of the ionic atmosphere of Cl^-, respectively. Surface charge density (σ) was, therefore, determined from $\Delta\psi$ according to the Gouy–Chapman theory, with a result that σ is nearly independent of I and proportional to X_{DC}. These results have led to a conclusion that the state of the diffuse layer surrounding the Stern layer at the micelle surface is represented well by the Gouy–Chapman theory.

Key words dibucaine hydrochloride; surface potential; mixed micelle; heptaethylene glycol dodecyl ether; Gouy–Chapman theory; diffuse layer

Dibucaine hydrochloride (DC) is a local anesthetic. It is important to study the surface activity of DC in connection with its pharmacological effects at the surface of a biological membrane, since a parallelism between surface activity and pharmacological potency has been proposed by Levy.[1] The surface tension of the aqueous solution of DC and the effect of sodium chloride on the critical micelle concentration (cmc) of DC have been studied.[2,3] Adrenergic drugs are administered to enhance the anesthetic effect of local anesthetics. I measured the surface tension of aqueous solutions of DC in the presence of adrenergic drugs and investigated the effects of adrenergic drugs on the cmc of DC. The value of cmc of DC decreased as the concentration of epinephrine hydrochloride (EPI) or phenylephrine hydrochloride (PHE) increased (the order of effect on the decrease in the cmc of DC was PHE > EPI > NaCl), and the surface tension of DC at the cmc (r_{cmc}) decreased with increasing concentrations of EPI or PHE.[4] The surface activity of DC was enhanced in the presence of adrenergic drugs: EPI and PHE were found to enhance the pharmacological effect of DC from the perspective of surface chemistry.

On the other hand, local anesthesia is known to be caused by local anesthetic molecules being incorporated into nerve membranes, which induce a change in the ion permeability of biological membranes by blocking sodium channels in the membranes. Local anesthetics have a tertiary amine group in the molecules. The anesthetic potency of local anesthetics may be dependent on the surface potential of the local anesthetic-nerve membrane mixed system. Therefore, investigating the surface potentials of local anesthetics and a nonionic surfactant (as a model of biological membrane) is of interest.

From these points of view, the surface potential of mixed micelle composed of DC and heptaethylene glycol dodecyl ether (HED) is measured, and the surface state of the mixed micelle is discussed.

Experimental

Materials Dibucaine hydrochloride (DC), heptaethylene glycol dodecyl ether (HED) as a nonionic surfactant, and ammonium 8-anilino-1-naphthalenesulfonate (ANS) as a fluorescent probe were purchased from Wako Pure Chemical Industries, Ltd. Mixed micelles composed of DC and HED were prepared, then dissolved in a buffer (pH 7.0 with an ionic strength of $0.02\,\mathrm{mol\cdot dm^{-3}}$) after which the fluorescence intensity was measured. All DC molecules were considered to form mixed micelles with HED on the basis of a previous result[5] that all sodium dodecyl sulfate (SDS) molecules formed mixed micelles with HED and also because DC is less soluble than SDS in a pH 7.0 buffer.

Measurement of Fluorescence Intensity Mixed micelles were dissolved in a buffer with an ionic strength of $0.02\,\mathrm{mol\cdot dm^{-3}}$ and a pH of 7.0. The total concentration of DC and HED was kept constant at $1\times10^{-3}\,\mathrm{mol\cdot dm^{-3}}$. A solution of ANS as a fluorescence probe was added to solutions of DC and HED up to $5\times10^{-5}\,\mathrm{mol\cdot dm^{-3}}$. The fluorescence intensity was measured by the use of a Hitachi F-4000 spectrofluorometer equipped with a pump-circulating water at a constant temperature through an outer tube. The temperature of the thermostat was maintained at 25±0.2 °C. The wavelengths of excitation and emission are 360 and 480 nm, respectively. Sodium chloride was used to control the ionic strength of the buffer solution. Louro *et al.*[6] reported that DC fluoresced at an emission wavelength of 420 nm when the aqueous solution of DC was excited at a wavelength of 328 nm. In this study, however, the fluorescence of DC did not disturb the measurement of ANS fluorescence intensity at 480 nm because the fluorescence intensity of DC at an emission wavelength of 480 nm when DC is excited at 360 nm is very weak, the concentration of DC is kept constant, and the fluorescence intensity of the DC-HED mixed system without ANS is treated as a blank value. The measured fluorescence intensity was corrected as described in a previous paper[5] based on the self-absorption of ANS at a higher concentration.

Results

Fluorescence Intensity The relationship between the fluorescence intensity, F, and the concentration of ANS, D, is shown in Fig. 1.

Adsorption of ANS at Micelle Surface It is considered that fluorescence intensity is proportional to the amount of ANS adsorbed at the micelle surface because ANS fluoresces when adsorbed at the micelle surface. The Langmuir adsorption isotherm can, therefore, be applied. The equation may be written as

$$\Gamma=\frac{D_m}{C}=\frac{\Gamma_s k D_f}{1+kD_f} \tag{1}$$

where Γ is the adsorbed amount of ANS per mole of micelle, Γ_s is the saturated adsorbed amount, k is the association constant related to the strength of adsorption,

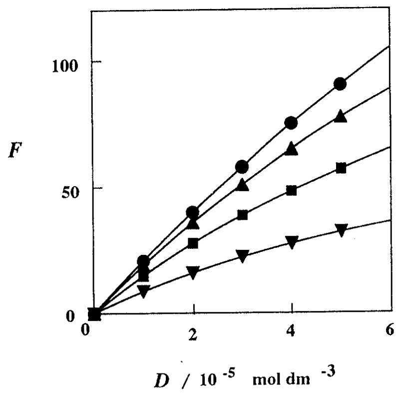

Fig. 1. Plots of Fluorescence Intensity *vs.* Concentration of ANS

X_{DC}: ●, 0; ▲, 0.25; ■, 0.50; ▼, 0.75. Ionic strength: 0.02 mol·dm^{-3}.

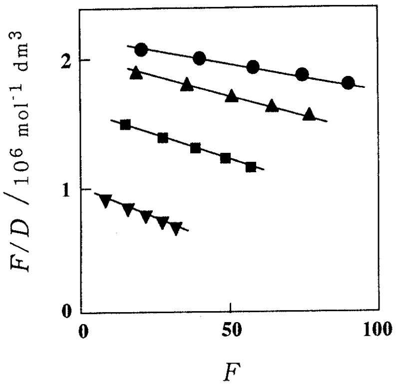

Fig. 2. Langmuir Plots of F/D *vs.* F

Symbols are the same as in Fig. 1. Ionic strength: 0.02 mol·dm^{-3}.

C is the total concentration of DC and HED, which are in the form of micelles, D_m is the concentration of bound ANS, and D_f is the concentration of free ANS; $D = D_m + D_f$. Fluorescence intensity F is related to D_m by

$$F = fD_m \tag{2}$$

where f is the fluorescence coefficient of ANS. F is defined by

$$F_\infty = \lim_{D \to \infty} F = Cf\Gamma_s \tag{3}$$

Equation 4 is, therefore, derived from Eqs. 1—3:

$$\frac{1}{F_\infty - F} = k\left(\frac{D}{F} - \frac{1}{f}\right) \tag{4}$$

Under the experimental conditions, D is sufficiently large. Therefore, Eq. 4 is reduced to

$$F/D = k(F_\infty - F) \tag{5}$$

Plots of F/D against F are presented in Fig. 2, and show a good linear relationship in accordance with Eq. 5. From the value of slope, k is evaluated.

Values of *k* The values of k are shown in Fig. 3 against the mole fraction of DC. The value of k decreased with an increasing mole fraction of DC. This is due to the electrostatic attraction between DC and ANS at the micelle surface.

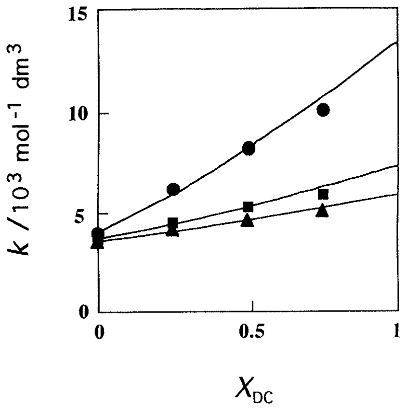

Fig. 3. Plots of k *vs.* Mole Fraction of DC

Ionic strength: ●, 0.02; ■, 0.1; ▲, 0.2.

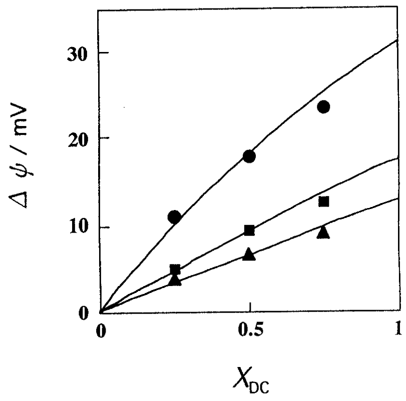

Fig. 4. Plots of Surface Potential of Micelle $\Delta\psi$ *vs.* Mole Fraction of DC

Symbols are the same as in Fig. 3. Solid lines: theoretical curves by the Gouy–Chapman equation for $\sigma = 1.338 \times 10^{-6} \cdot X_{DC}$ (c cm^{-2}).

Discussion

Surface Potential of Micelles On the assumption described in a previous paper,[5] the binding constant of ANS to DC–HED mixed micelles, k, depends on the surface potential of the micelle, $\Delta\psi$. The relation is, therefore, written as

$$k = k_0 \exp(-zF_A\Delta\psi/\boldsymbol{R}T) \tag{6}$$

where z is the valency of ANS, F_A is the Faraday constant, $\boldsymbol{R}$ is the gas constant, and k_0 is the k for nonionic HED micelle. By substituting $z = -1$, $F_A = e \cdot N_A = 9.648 \times 10^4$ (c mol^{-1}), $\boldsymbol{R} = 8.314$ (J·mol^{-1}·K^{-1}), and $T = 298.15$ K at 25 °C, I obtained

$$\Delta\psi = -59.16 \cdot \log(k_0/k) \tag{7}$$

The surface potential of the micelle, $\Delta\psi$, as calculated from the values of k shown in Fig. 3 by using Eq. 7, is plotted against the mole fraction of DC in Fig. 4.

To investigate the effect of ionic strength on the surface potential of the micelle, experiments were carried out with buffer solutions containing sodium chloride so that their ionic strengths are 0.10 and 0.20 mol·dm^{-3} as well as 0.02 mol·dm^{-3}, with plots of $\Delta\psi$ against the mole fraction of DC represented by the symbols ■ and ▲ in Fig. 4.

No micelles composed of DC molecules alone are formed under these experimental conditions, in which the total

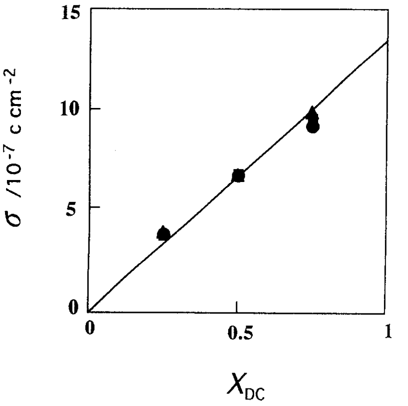

Fig. 5. Plots of Surface Charge Density σ *vs.* Mole Fraction of DC

Symbols are the same as in Fig. 3.

concentration of DC and HED is $1\times10^{-3}\,\text{mol}\cdot\text{dm}^{-3}$. The critical micelle concentration of DC in water at 25 °C is about $74\times10^{-3}\,\text{mol}\cdot\text{dm}^{-3}$.[4] The surface potential of a DC micelle is, therefore, obtained by extrapolation of the theoretical curve based on the Gouy–Chapman equation, which will be described in a later section. The surface potential of DC micelle is +31 mV at an ionic strength of $0.02\,\text{mol}\cdot\text{dm}^{-3}$ (+17.5 and 12.7 mV at ionic strengths of 0.10 and $0.20\,\text{mol}\cdot\text{dm}^{-3}$, respectively). These values for DC micelles are less than the value[7] (+64 mV at ionic strength $0.10\,\text{mol}\cdot\text{dm}^{-3}$) for dodecyltrimethylammonium chloride micelles as a general cationic surfactant, which suggests that DC forms a loosely packed micelle.

The surface potential of the micelle becomes more positive with an increasing mole fraction of DC. On the other hand, the surface potential of the micelle becomes smaller as the ionic strength is increased by adding sodium chloride. These changes may be considered to be due to the effects of the positive charge of DC and of the ionic atmosphere of Cl^-, respectively.

Surface Charge Density of Micelles According to the Gouy–Chapman theory, the relation between surface potential, $\Delta\psi$, and surface charge density, σ, is expressed as

$$\frac{\sigma}{c\,\text{cm}^{-2}}=5.8673\times10^{-6}\cdot\sqrt{\sum_i\left[\left(\frac{C_i}{\text{mol dm}^{-3}}\right)\left\{\exp\left(-0.03892\,z_i\cdot\frac{\Delta\psi}{\text{mV}}\right)-1\right\}\right]} \tag{8}$$

where 78.54[8] is used as the value for the relative dielectric constant of water at 25 °C. The relationship between surface charge density and the mole fraction of DC is shown in Fig. 5.

The value of σ was found to be nearly proportional to X_{DC}, and thus the relation can be written as

$$\sigma=1.338\times10^{-6}\cdot X_{DC}\,, \tag{9}$$

although the value of σ shifted slightly downward from the straight line with an increasing mole fraction of DC because of the smaller ionic dissociation of DC at the higher mole fraction of DC. By using Eqs. 7—9, theoretical values of the surface potential of the micelles and the binding constant were calculated, with the results shown in Figs. 3 and 4 by solid lines, respectively. The surface charge density, σ, was found to be nearly independent of ionic strength. These results indicate that the state of the diffuse layer surrounding the Stern layer at the micelle surface is represented well by the Gouy–Chapman theory.

Conclusion

The surface potential of mixed micelles composed of DC and HED has been determined by the fluorescence method. The surface potential of the micelle increases with an increasing mole fraction of DC (X_{DC}), and decreases with increasing ionic strength. The surface state of the micelle is represented well by the Gouy–Chapman theory.

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