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Dissociation equilibrium between uncharged and charged local anesthetic lidocaine in a surface-adsorbed film

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Abstract The dissociation equilibrium between uncharged local anesthetic lidocaine (LC) and charged local anesthetic LC ($LC \bullet H^+$) in a surface-adsorbed film was investigated by measuring the surface tension and pH of aqueous solutions of a mixture of hydrochloric acid and LC. The surface tension values decreased slightly with increasing total molality m_t at $0 \le X_2 \le 0.5$, where X_2 is the mole fraction of LC in the mixture, while they decreased rapidly with increasing m_t at $0.5 < X_2 \le 1$. It was shown from the pH measurements that almost all LC molecules were changed into $LC \bullet H^+$ ions by protonation at $0 \le X_2 \le 0.5$ and both forms coexisted only at $0.5 < X_2 \le 1$. The quantities of the respective LC and $LC \bullet H^+$ transferred from the aqueous solution to the adsorbed film, i.e., their surface densities, were calculated by applying the thermodynamic equations derived to the surface tension and pH data. A greater quantity of LC than $LC \bullet H^+$ existed in the adsorbed film at the coexisting composition. The partitioning behavior of LC and $LC \bullet H^+$ in the adsorbed film was characterized by three composition regions: (1) slight partitioning of low surface-active $LC \bullet H^+$ in the region at $0 \le X_2 \le 0.5$, (2) preferential partitioning of LC at $0.5 < X_2 <$ around 0.7, and (3) negative partitioning of $LC \bullet H^+$ at around $0.7 \le X_2 \le 1$. The present results clearly indicate that uncharged local anesthetics transfer into hydrophobic environments such as cell membranes more than charged ones.

Keywords Surface tension · Surface-adsorbed film · Uncharged local anesthetic · Charged local anesthetic · Preferential partitioning · Negative adsorption

Introduction

Local anesthetics are cationic surfactants of tertiary amine forms with an aromatic ring. They have relatively high p K_a values in the range 7.5–9.0 [1, 2, 3]. They exist as a mixture of positively charged and neutral (uncharged) species depending on the pH of the aqueous solution. Despite several reports about the effects of local anesthetics on cell and/or model membranes [4, 5, 6, 7], the ratio between uncharged and charged anesthetics in aqueous solution is not accurately described in many studies, much less the ratio of both anesthetics in the membrane. The ratio of uncharged to charged anesthetics in aqueous solution is determined by the p K_a value and pH value of the solution. On the other hand, it is considerably difficult to know the ratio of those partitioned into the membrane. This difficulty arises because there is no restraint in the quantities of the species partitioned into the membrane so long as the condition of electroneutrality holds only in the membrane.

Surface-adsorbed films produce microscopic environments with hydrophobicity similar to biomembranes. The thermodynamic behavior of adsorbed films can be determined accurately from surface tension data [8, 9]. It is advantageous theoretically and experimentally to use the adsorbed film as a model membrane for the study of drug partitioning into the membrane [10, 11, 12]. The dissociation equilibrium between uncharged and charged local anesthetics in the adsorbed film can be elucidated by examining the mixed surface adsorption of both anesthetics under the condition in which they coexist in aqueous solution. Since they coexist at physiological pH, the surface activities are useful for the study of the molecular mechanism of local anesthesia.

In the present study, we are concerned with a mixture of a strong acid and an uncharged local anesthetic because the coexistent state of uncharged and charged anesthetics can be prepared without using a buffer solution and the surface properties of uncharged local anesthetics were previously reported [13]. A mixture of hydrochloric acid (HCl) and uncharged local anesthetic lidocaine (LC) was selected for this purpose. We produced the coexisting state of LC and the charged LC cation (LC•H$^+$) by mixing HCl with LC in aqueous solution. The surface tension and the pH of aqueous solutions of the HCl–LC mixture were measured. The quantities of uncharged and charged anesthetics partitioned into the adsorbed film were evaluated by thermodynamic equations. The dissociation equilibrium between LC and LC•H$^+$ in the adsorbed film is discussed.

Theoretical

One way to prepare the coexistent state of uncharged and charged local anesthetics is to mix the uncharged anesthetic with a strong acid like HCl. Alternatively, one can mix the charged anesthetic with a strong base, for example, sodium hydroxide. Local anesthetics in clinical use are hydrochloride salts and some ester-type charged anesthetics such as tetracaine and procaine hydrochlorides are hydrolyzed rapidly in the presence of a strong base [13, 14]. It is reasonable to use a mixture of an amide-type uncharged anesthetic and an acid. Here we derive the thermodynamic equations applicable to the former mixture, which are based on the surface thermodynamics of Motomura et al. [15, 16] and Ikeda and Ozeki [17, 18], in order to analyze the mixed surface-adsorbed films of uncharged and charged anesthetics.

Let us consider a system composed of air, water, HCl, and uncharged local anesthetic A. Here HCl is a strong electrolyte, while A is a weak electrolyte: HCl completely dissociates into a proton and a chloride ion and A can change into an AH$^+$ ion (charged local anesthetic) by protonation from HCl by the following reactions:

$$\mathrm{HCl} \Leftrightarrow \mathrm{H}^{+} + \mathrm{Cl}^{-} \tag{1}$$

and

$$\mathrm{A} + \mathrm{H}^{+} \Leftrightarrow \mathrm{AH}^{+}. \tag{2}$$

The surface tension γ is expressed as a function of the chemical potential μ_i of species i at constant temperature T and pressure p [18]:

$$\begin{aligned} -\mathrm{d}\gamma &= \Gamma_{\mathrm{H_2O}}\mathrm{d}\mu_{\mathrm{H_2O}} + \Gamma_{\mathrm{H^+}}\mathrm{d}\mu_{\mathrm{H^+}} + \Gamma_{\mathrm{OH^-}}\mathrm{d}\mu_{\mathrm{OH^-}} \\ &\quad + \Gamma_{\mathrm{A}}\mathrm{d}\mu_{\mathrm{A}} + \Gamma_{\mathrm{AH^+}}\mathrm{d}\mu_{\mathrm{AH^+}} + \Gamma_{\mathrm{Cl^-}}\mathrm{d}\mu_{\mathrm{Cl^-}} + \Gamma_{\mathrm{air}}\mathrm{d}\mu_{\mathrm{air}}, \end{aligned} \tag{3}$$

where Γ_i is the surface excess number of moles per unit area of species i . The ionization equilibrium and the stoichiometric relations in the aqueous solution are represented as

$$\mu_{\mathrm{H_2O}} = \mu_{\mathrm{H^+}} + \mu_{\mathrm{OH^-}}, \tag{4}$$

$$\mu_{\mathrm{AH^+}} = \mu_{\mathrm{A}} + \mu_{\mathrm{H^+}}, \tag{5}$$

and

$$\mu_{\mathrm{HCl}} = \mu_{\mathrm{H^+}} + \mu_{\mathrm{Cl^-}}, \tag{6}$$

respectively, and the electroneutrality condition among surface excess values of ionic species is given by

$$\Gamma_{\mathrm{H^+}} + \Gamma_{\mathrm{AH^+}} = \Gamma_{\mathrm{OH^-}} + \Gamma_{\mathrm{Cl^-}}. \tag{7}$$

Substitution of Eqs. (4), (5), (6), and (7) into Eq. (3) leads us to the equation

$$\begin{aligned} -\mathrm{d}\gamma &= (\Gamma_{\mathrm{H_2}}\mathrm{O} + \Gamma_{\mathrm{OH^-}})\mathrm{d}\mu_{\mathrm{H_2}}\mathrm{O} + \Gamma_{\mathrm{air}}\mathrm{d}\mu_{\mathrm{air}} \\ &\quad + (\Gamma_{\mathrm{A}} + \Gamma_{\mathrm{AH^+}})\mathrm{d}\mu_{\mathrm{A}} + \Gamma_{\mathrm{Cl^-}}\mathrm{d}\mu_{\mathrm{HCl}} \end{aligned} \tag{8}$$

Now we specify the surface excess thermodynamic quantities with respect to the two dividing planes defined by [19]

$$\Gamma_{\mathrm{H_2O}} + \Gamma_{\mathrm{OH^-}} = 0 \tag{9}$$

and

$$\Gamma_{\mathrm{air}} = 0, \tag{10}$$

where the convention of Eq. (9) comes from the fact that the surface excess value of added water is equal to the sum of the values for unionized water and hydroxide ion in the aqueous solution [17, 18]. Then, we arrive at the adsorption equation described in terms of the chemical potentials of the solute component:

$$-\mathrm{d}\gamma = \left(\Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH^+}}\right)\mathrm{d}\mu_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{Cl^-}}\mathrm{d}\mu_{\mathrm{HCl}}, \tag{11}$$

where the superscript H is used to stress the way of selecting the dividing planes defined by Eqs. (9) and (10). Assuming the ideality of the solution, we obtain the following equation:

$$-\,\mathrm{d}\gamma = \left(\Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH}^{+}}\right) RT\mathrm{dln}m_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{Cl}^{-}} RT\mathrm{dln}m_{\mathrm{H}^{+}}m_{\mathrm{Cl}^{-}}, \tag{12}$$

where m_i is the molality of species i present in the solution. We consider the dissociation equilibrium of anesthetic and water to describe Eq. (12) by using experimental variables, i.e., overall concentrations of HCl (m_1) and anesthetic (m_2). If the degree of protonation of the anesthetic is α, stoichiometric relations holds as

$$m_{\mathrm{AH}^{+}} = \alpha m_2, \tag{13}$$

$$m_{\mathrm{A}} = (1 - \alpha) m_2, \tag{14}$$

and

$$m_{\mathrm{Cl}^{-}} = m_1. \tag{15}$$

The acid dissociation constant of the protonated anesthetic (K_{a}) and the ionic product of water (K_{w}) are, respectively, defined by

$$K_{\mathrm{a}} = [(1-\alpha)/\alpha] m_{\mathrm{H}^{+}} \tag{16}$$

and

$$K_{\mathrm{w}} = m_{\mathrm{H}^{+}} m_{\mathrm{OH}^{-}}. \tag{17}$$

The electroneutrality condition in the aqueous solution is given by

$$m_{\mathrm{H}^{+}} + m_{\mathrm{AH}^{+}} = m_{\mathrm{OH}^{-}} + m_{\mathrm{Cl}^{-}}. \tag{18}$$

Noting the dependence of α on m_1 and m_2, respectively, given by

$$(\partial\alpha/\partial m_1)_{T,p,m_2} = [\alpha(1-\alpha)]/Q \tag{19}$$

and

$$(\partial\alpha/\partial m_2)_{T,p,m_1} = -\left[\alpha^2(1-\alpha)\right]/Q, \tag{20}$$

where

$$Q = m_1 - \alpha^2 m_2 + 2m_{\mathrm{OH}^{-}} = \alpha(2 - \alpha) m_2 - m_1 + 2m_{\mathrm{H}^{+}}, \tag{21}$$

and arranging Eq. (12) by using Eqs. (13), (14), (15), (16), (17), (18), (19), (20), and (21), we can obtain the equation

$$-\,\mathrm{d}\gamma = \Gamma^{\mathrm{H}}_{1} RT\mathrm{dln}m_1 + \Gamma^{\mathrm{H}}_{2} RT\mathrm{dln}m_2, \tag{22}$$

where

$$\Gamma^{\mathrm{H}}_{1} = -\left(\Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH}\ +}\right)\alpha m_1 \Big/ Q + \Gamma^{\mathrm{H}}_{\mathrm{Cl}}\text{-}\,(1 + m_1/Q) \tag{23}$$

and

$$\Gamma^{\mathrm{H}}_{2} = \left(\Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH}\ +}\right)(m_1 + 2m_{\mathrm{OH}}\text{-}\,) \Big/ Q - \Gamma^{\mathrm{H}}_{\mathrm{Cl}}\text{-}\,\alpha m_2/Q. \tag{24}$$

Here Γ^{H}_{1} and Γ^{H}_{2} are the parameters which can be obtained from the derivatives of γ with respect to m_1 and m_2.

It has been shown that the total molality of the mixture (m_{t}) and the mole fraction in the total components (X_2) are advantageous independent variables in examining the adsorption behavior of a two-component system from the surface tension data thermodynamically [15, 16]. So we chose m_{t} and X_2 as concentration variables defined, respectively, by

$$m_{\mathrm{t}} = m_1 + m_2 \tag{25}$$

and

$$X_2 = m_2/m_{\mathrm{t}}. \tag{26}$$

Transforming the variables m_1 and m_2 in Eq. (22) into m_{t} and X_2 by using Eqs. (25) and (26), we can write Eq. (22) in another differential form as

$$\begin{aligned} -\,\mathrm{d}\gamma = {} & \left(RT\Gamma^{\mathrm{H}}_{\mathrm{t}}/m_{\mathrm{t}}\right)\left(AX^{\mathrm{H}}_{1} + BX^{\mathrm{H}}_{2}\right)\mathrm{d}m_{\mathrm{t}} \\ & - RT\Gamma^{\mathrm{H}}_{\mathrm{t}}\left[C\left(X^{\mathrm{H}}_{1}/X_1\right) - D\left(X^{\mathrm{H}}_{2}/X_2\right)\right]\mathrm{d}X_2, \end{aligned} \tag{27}$$

where we introduced the total surface density of the mixture ($\Gamma^{\mathrm{H}}_{\mathrm{t}}$) and the mole fraction in the adsorbed film (X^{H}_{1}, X^{H}_{2}) defined by

$$\Gamma^{\mathrm{H}}_{\mathrm{t}} = \Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH}\ +} + \Gamma^{\mathrm{H}}_{\mathrm{Cl}}\text{-}\ , \tag{28}$$

$$X^{\mathrm{H}}_{1} = \Gamma^{\mathrm{H}}_{\mathrm{Cl}^{-}}/\Gamma^{\mathrm{H}}_{\mathrm{t}} = \left(\Gamma^{\mathrm{H}}_{\mathrm{AH}^{+}} + \Gamma^{\mathrm{H}}_{\mathrm{H}^{+}} - \Gamma^{\mathrm{H}}_{\mathrm{OH}^{-}}\right)/\Gamma^{\mathrm{H}}_{\mathrm{t}}, \tag{29}$$

and

$$X^{\mathrm{H}}_{2} = \left(\Gamma^{\mathrm{H}}_{\mathrm{A}} + \Gamma^{\mathrm{H}}_{\mathrm{AH}^{+}}\right)/\Gamma^{\mathrm{H}}_{\mathrm{t}}, \tag{30}$$

respectively. The quantities A, B, C, and D are the thermodynamic parameters determined as a function of α, m_{t}, and X_2, which are defined, respectively, by

$$A = 1 - [(\alpha X_2 - X_1)m_{\mathrm{t}}]/Q, \tag{31}$$

$$B = 1 + [\alpha(\alpha X_2 - X_1)m_{\mathrm{t}}]/Q, \tag{32}$$

$$C = 1 + [(1+\alpha)m_{\mathrm{t}}X_1]/Q, \tag{33}$$

and

$$D = 1 + [\alpha(1+\alpha)m_{\mathrm{t}}X_2]/Q. \tag{34}$$

From Eq. (27), we have

$$\begin{aligned} & \left(\frac{\partial m_{\mathrm{t}}}{\partial X_2}\right)_{T,p,\gamma} \\ & = \left\{\left[C\left(X^{\mathrm{H}}_{1}/X_1\right) - D\left(X^{\mathrm{H}}_{2}/X_2\right)\right]m_{\mathrm{t}}\right\}/\left(AX^{\mathrm{H}}_{1} + BX^{\mathrm{H}}_{2}\right). \end{aligned} \tag{35}$$

Then the value of X^{H}_{2} is expressed as

$$X^{\mathrm{H}}_{2} = (AZ - X_2C)/[(D - C)X_2 + (A - B)Z - D], \tag{36}$$

where Z is given by

$$Z = (X_1X_2/m_{\mathrm{t}})(\partial m_{\mathrm{t}}/\partial X_2)_{T,p,\gamma}. \tag{37}$$

The surface quantity $\Gamma_{\mathrm{t}}^{\mathrm{H}}$ is derived from Eq. (27) by

$$\Gamma_{\mathrm{t}}^{\mathrm{H}} = -\left\{m_{\mathrm{t}}/\left[RT\left(AX_1^{\mathrm{H}} + BX_2^{\mathrm{H}}\right)\right]\right\}(\partial\gamma/\partial m_{\mathrm{t}})_{T,p,X_2}. \tag{38}$$

Therefore, the values of X_2^{H} and $\Gamma_{\mathrm{t}}^{\mathrm{H}}$ can be calculated by applying Eqs. (36) and (38) to the surface tension and pH data measured as a function of m_{t} and X_2.

In the absence of HCl, Eq. (38) with the relations, $m_{\mathrm{t}}=m_2$ and $X_2 = X_2^{\mathrm{H}} = 1$, we have the equation

$$\Gamma_{\mathrm{t}}^{\mathrm{H}} = -\left\{m_2/\left[RT\left(1 - \alpha^2 m_2/2m_{\mathrm{OH}^-}\right)\right]\right\}(\partial\gamma/\partial m_2)_{T,p}. \tag{39}$$

If α is significantly small, Eq. (39) corresponds the adsorption isotherm for a nonionic substance. On the other hand, in the absence of local anesthetic, Eq. (38) with the relations, $m_{\mathrm{t}}=m_1$, $X_2 = X_2^{\mathrm{H}} = 0$, and $m_{\mathrm{OH}}{}^{-}\approx 0$, we have

$$\Gamma_{\mathrm{t}}^{\mathrm{H}} = -(m_1/2RT)(\partial\gamma/\partial m_1)_{T,p}. \tag{40}$$

This equation corresponds to the isotherm for a uni-univalent electrolyte.

The total quantities of adsorbed anesthetics can be obtained from the values of X_2^{H} and $\Gamma_{\mathrm{t}}^{\mathrm{H}}$; however, we cannot obtain the ratio of uncharged anesthetic to charged anesthetic in the adsorbed film. To estimate the contributions of both anesthetics in the adsorbed film, it will be necessary to measure some electric properties such as the surface potential or the surface conductivities or to make some postulates. The latter method was used in this study. We now introduce a nonthermodynamic assumption [20, 21]:

$$\Gamma_{\mathrm{H}^+}^{\mathrm{H}} = \Gamma_{\mathrm{OH}^-}^{\mathrm{H}}. \tag{41}$$

This assumption says that the surface layer of water is always neutral. Although the assumption of Eq. (41) may not be correct quantitatively, it is significant that the difference between Γ_{H^+} and Γ_{OH^-} is very small because the proton and the hydroxyl ion are surface-inactive like inorganic salts. Accordingly, it is significant that the results obtained by use of Eq. (41) are correct qualitatively. Without added HCl, Eqs. (7) and (28) become

$$\Gamma_{\mathrm{AH}}^{\mathrm{H}}\ + \ = 0 \tag{42}$$

and

$$\Gamma_{\mathrm{A}}^{\mathrm{H}} = \Gamma_{\mathrm{t}}^{\mathrm{H}}. \tag{43}$$

With added HCl, Eq. (7) becomes

$$\Gamma_{\mathrm{AH}^+}^{\mathrm{H}} = \Gamma_{\mathrm{Cl}^-}^{\mathrm{H}}. \tag{44}$$

We can separate the adsorption density of the anesthetic into those of uncharged and charged forms under the condition. From Eqs. (29) and (30)

$$\Gamma_{\mathrm{AH}^+}^{\mathrm{H}} = \Gamma_{\mathrm{t}}^{\mathrm{H}}X_1^{\mathrm{H}} \tag{45}$$

and

$$\Gamma_{\mathrm{A}}^{\mathrm{H}} = \Gamma_{\mathrm{t}}^{\mathrm{H}}\left(X_2^{\mathrm{H}} - X_1^{\mathrm{H}}\right), \tag{46}$$

respectively. It is approximately possible to consider the ratio of both forms in the adsorbed film in terms of Eqs. (45) and (46).

Experimental

Materials

LC [2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide] was obtained as its free base form from Sigma Chemical Co. (St. Louis, MO, USA) and prepared by recrystallizing it three times from an ethanol and water mixture. Its purity was checked by elemental analysis. HCl (Suprapur reagent 30%) was purchased from Merck (Darmstadt, Germany) and used directly without further purification. Preliminary measurements of the surface tension of both solutions showed no time dependence for the adsorption equilibrium values of the surface tension. A schematic drawing of the protonation of LC by HCl performed in this study and the fraction for uncharged and charged forms of LC in the solution as a function pH at 298.15 K are illustrated in Fig. 1. Water was triply distilled after deionization treatment, where the second and third distillations were done from dilute alkaline permanganate solution.

Automatic surface tension measurements

The ratio of LC to LC•H$^+$ in the surface-adsorbed film was estimated from the surface tension data. γ of the aqueous solutions of HCl and LC mixture was measured by an automatic system based on the drop-volume method (Yamashita Giken Co., Tokushima, Japan) [22, 23] as a function of m_{t} and X_2, which were defined in the previous section. All measurements were carried out at 298.15 ± 0.01 K by immersing the measurement unit in a thermostated water bath under atmospheric pressure. The experimental error for the value of the surface tension was less than 0.08 mN m^{-1}.

pH measurements and determination of HCl molality

The pH of the aqueous HCl–LC solutions was measured using a pH meter (Denki Kagaku Kogyo, PHL-40) in a

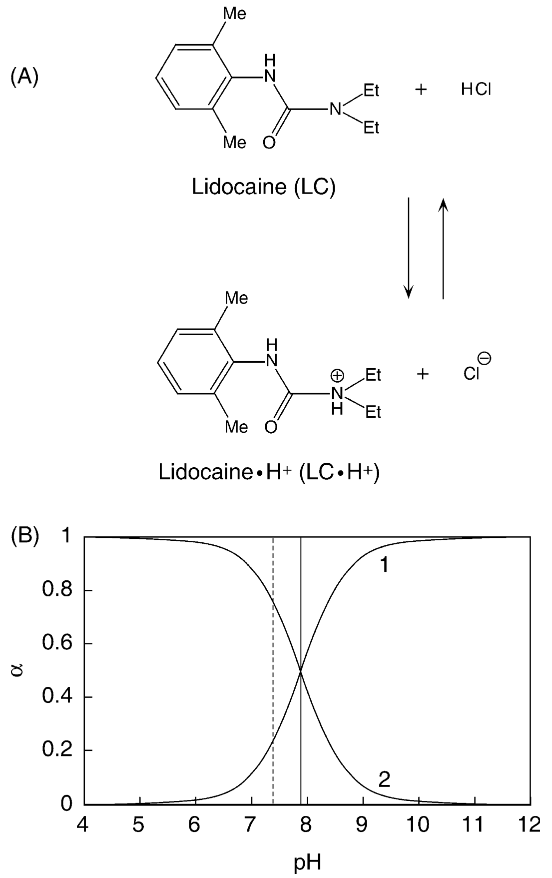

Fig. 1 **A** Protonation of uncharged local anesthetic lidocaine (LC) by hydrochloric acid. **B** Fraction for uncharged and charged forms of LC in aqueous solution as a function pH at 298.15 K: LC (*1*), LC•H$^+$ (*2*). The *broken* and *solid lines* correspond to pH 7.4 and pK_a, respectively

thermostated water bath maintained at a temperature of 298.15 ± 0.2 K under the same experimental conditions as for the surface tension measurements so as to determine the ratio of LC to LC•H$^+$ in the aqueous solution. The molarity of the aqueous HCl solution (m_1) was estimated from its molar concentration (c_1), which was determined from potentiometric titration of sodium carbonate (99.98%, Asahi Glass Company). The m_1 value was converted from the c_1 value by use of the molecular weight of HCl and the solution density. The density was evaluated from the apparent molar volume reported in the literature [24]. By adding uncharged anesthetic into a HCl solution with a known m_1 value, stock solutions with constant X_2 values were made and were diluted to prepare the sample solutions.

Results

The experimental results of the surface tension measurements for the HCl–LC mixture are shown in Fig. 2.

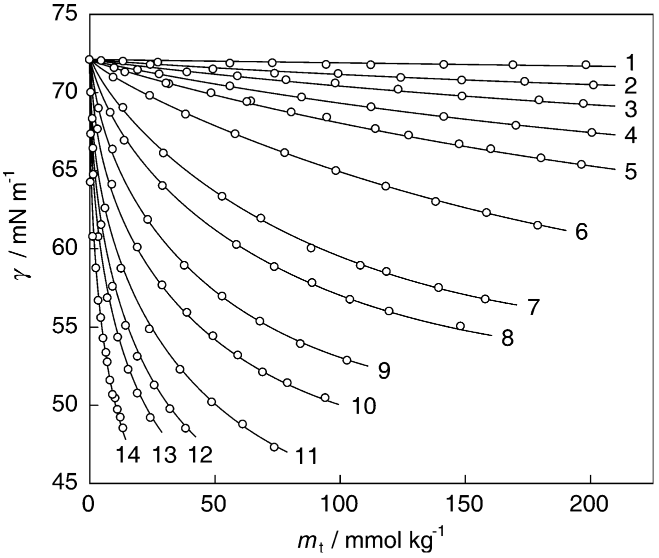

Fig. 2 Surface tension versus total molality curves at constant composition: $X_2 = 0$ (HCl) (*1*), 0.080 (*2*), 0.100 (*3*), 0.250 (*4*), 0.500 (*5*), 0.502 (*6*), 0.505 (*7*), 0.510 (*8*), 0.525 (*9*), 0.550 (*10*), 0.600 (*11*), 0.675 (*12*), 0.750 (*13*), 1 (LC) (*14*)

They are illustrated in the form of γ versus m_t curves at constant X_2. The γ values of pure HCl ($X_2 = 0$) did not vary with m_t and they have virtually the same γ values as pure water (71.96 mN m^{-1}), whereas those of pure LC ($X_2 = 1$) decreased steeply with a slight increase in m_t. The LC solution reached the solubility limit at about 14 mmol kg^{-1} [13]. In the mixture, the γ versus m_t curves at small compositions showed remarkably different behavior from those at large compositions. The curves significantly changed just beyond the equimolar composition ($X_2 = 0.5$) and the composition formed a boundary of the variation. The γ values did not appreciably decrease with increasing m_t in the former composition range, while a rapid decrease in γ was observed and the solution reached its solubility limit in the latter composition range.

The results of pH measurements for the mixtures are shown in Fig. 3. Although the pH value of each composition became almost constant with increasing m_t, the pH versus m_t curves were significantly dependent on X_2 in a similar manner as the γ versus m_t curves in Fig. 2. The pH values at small compositions had values of less than 3, while those at large compositions had values of more than 6; the pH value of pure HCl was not depicted in the figure because of its negative value. The ratio of LC to LC•H$^+$ in the aqueous solution, i.e., the value of α estimated from the results of pH measurements by using Eq. (16) (the so-called Henderson–Hasselbalch equation), is given in the following.

$$\mathrm{pH} = \mathrm{p}K_a + \log[(1 - \alpha)/\alpha] \qquad (47)$$

The resulting α versus m_t curves at constant X_2 are shown in Fig. 4, where the value of 7.92 for the p K_a values of LC at 298.15 K was taken from the literature [1]. The α values were 1 at small compositions less than $X_2 = 0.5$. This means that the uncharged LC molecules completely changed into the cationic LC•H^+ by protonation from HCl and there is no uncharged form of LC at $0 \leq X_2 \leq 0.5$. On the other hand, they varied between 0 and 1 at large composition where $X_2 > 0.5$. Therefore, the LC and LC•H^+ molecules coexist only at $0.5 < X_2 \leq 1$. It is noticed from the α versus m_t curve of pure LC that almost all the molecules exist as uncharged forms in the pure LC solution. We discuss the competitive adsorption behavior of LC and LC•H^+ in the coexistent composition region by using the thermodynamic equations derived in the previous section.

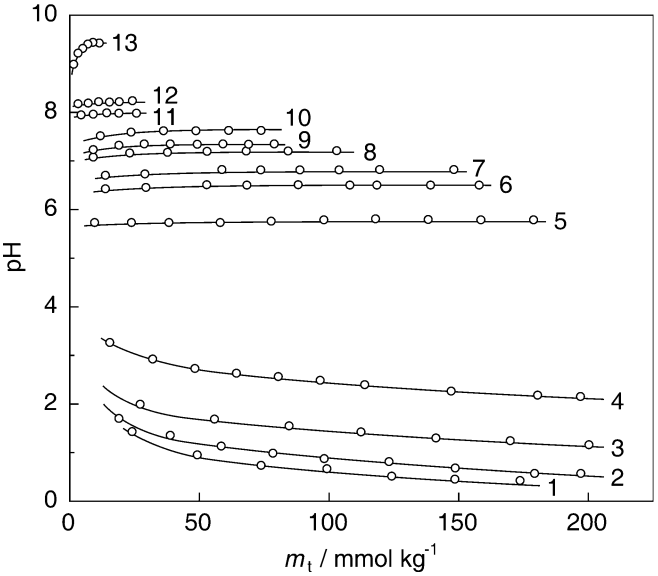

Fig. 3 pH versus total molality curves at constant composition: $X_2 = 0.080$ (*1*), 0.100 (*2*), 0.250 (*3*), 0.500 (*4*), 0.502 (*5*), 0.505 (*6*), 0.510 (*7*), 0.525 (*8*), 0.550 (*9*), 0.600 (*10*), 0.675 (*11*), 0.750 (*12*), 1 (LC) (*13*)

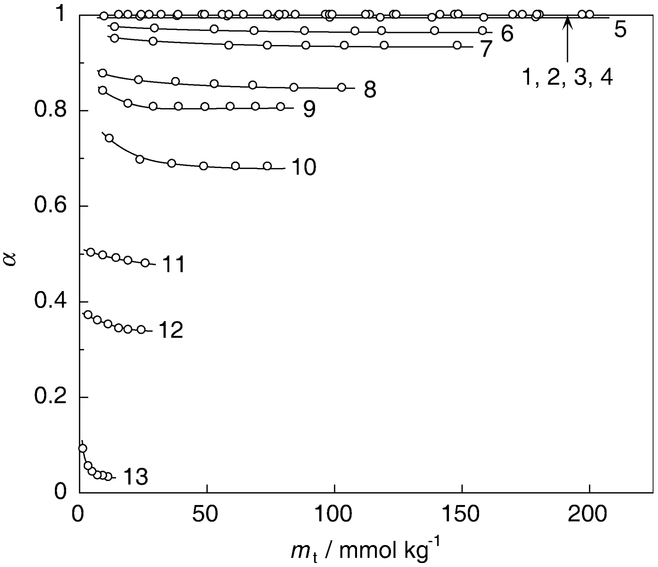

Fig. 4 Degree of dissociation for lidocaine versus total molality curves at constant composition: $X_2 = 0.080$ (*1*), 0.100 (*2*), 0.250 (*3*), 0.500 (*4*), 0.502 (*5*), 0.505 (*6*), 0.510 (*7*), 0.525 (*8*), 0.550 (*9*), 0.600 (*10*), 0.675 (*11*), 0.750 (*12*), 1 (LC) (*13*)

Discussion

In order to analyze the dissociation equilibrium in the adsorbed film, the partitioning quantities of LC and LC•H^+ into the adsorbed film must be evaluated. These are estimated from the surface densities defined by Eqs. (45) and (46). For the evaluation of these surface densities, it is necessary to obtain the values of Γ_t^H and X_2^H defined by Eqs. (28) and (30).

Let us first determine the value of X_2^H by using Eq. (36). LC coexists with LC•H^+ only in the composition range above $X_2 = 0.5$ in this study. Therefore, we focus our attention on this composition range. The m_t versus X_2 curves at constant γ, which were constructed by selecting the m_t values at a given γ in the coexistent composition range, are illustrated in Fig. 5. The m_t values increased with decreasing X_2. The lower the γ values, the steeper the slopes of the m_t versus X_2 curves became. The Q value of Eq. (21) was obtained by using the m_t versus X_2 curve and the α versus m_t curve in

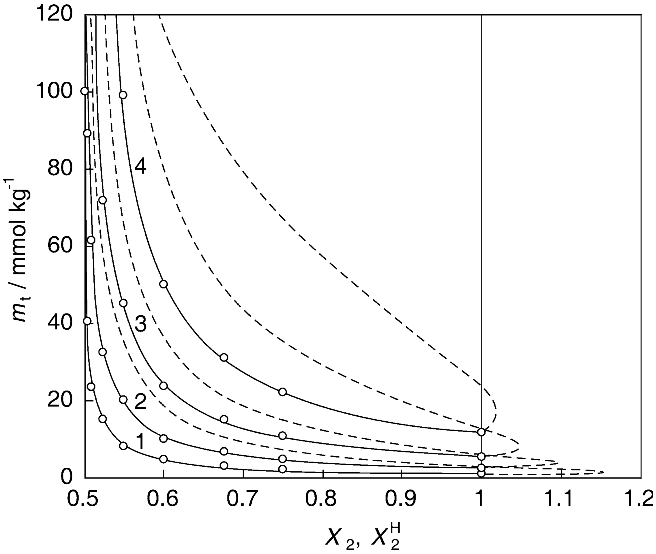

Fig. 5 Total molality versus composition curves at constant surface tension in the coexistent composition range of uncharged and charged LC: $\gamma = 65$ mN m^{-1} (*1*), 60 (*2*), 55 (*3*), 50 (*4*); m_t versus X_2 (*solid line*), m_t versus X_2^H (*broken line*)

Fig. 4. The thermodynamic parameters A, B, C, and D defined by Eqs. (31), (32), (33), and (34), respectively, were calculated. Substituting the previous parameters with the Z value, which was estimated by applying Eq. (37) to the m_t versus X_2 curve, into Eq. (36), we obtained the X_2^H value. The m_t versus X_2^H curves are drawn as broken lines together with the corresponding m_t versus X_2 curves in Fig. 5. Although the X_2^H values were larger than the X_2 values, we immediately recognize that the m_t versus X_2^H curves behave peculiarly; the X_2^H value exceeds unity at a high X_2 value and then steeply approaches 0.5 with decreasing X_2 because of X_2 is approximately equal to X_2^H at $X_2 = 0.5$. Considering the definition of X_2^H in Eq. (30), the peculiar behavior is attributable to the negative value of $\Gamma^H_{AH^+}$ in the high X_2 region as explained later.

The values of Γ_t^H were estimated by using the X_2^H value obtained, the slope of the γ versus m_t curve in Fig. 2, and the A and B values with Eq. (38). The variation in Γ_t^H with m_t at constant X_2 is shown in Fig. 6. The Γ_t^H value increased with increasing m_t and X_2. The behavior of the Γ_t^H versus m_t curves in the low and high composition ranges is appreciably different as expected from the γ versus m_t curve in Fig. 2. HCl is a surface-inactive electrolyte and does not adsorb into the surface layer. In contrast, uncharged LC is highly surface active. Because the LC molecules do not exist at $0 \leq X_2 \leq 0.5$, we may say from the comparison of the Γ_t^H values at low X_2 with those at high X_2 that the surface activity of LC is much higher than that of LC•H$^+$.

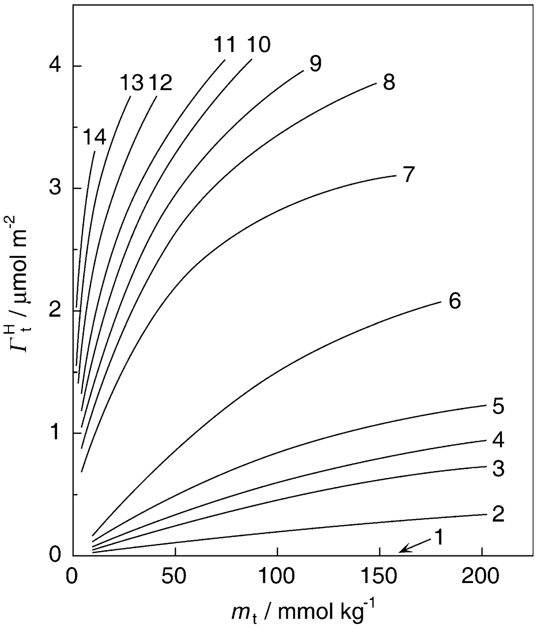

Fig. 6 Total surface density versus total molality curves at constant composition: $X_2 = 0$ (HCl) (*1*), 0.080 (*2*), 0.100 (*3*), 0.250 (*4*), 0.500 (*5*), 0.502 (*6*), 0.505 (*7*), 0.510 (*8*), 0.525 (*9*), 0.550 (*10*), 0.600 (*11*), 0.675 (*12*), 0.750 (*13*), 1 (LC) (*14*)

We now examine the respective surface densities of LC, Γ_A^H, and LC•H$^+$, $\Gamma^H_{AH^+}$, by using the X_2^H and Γ_t^H values obtained with Eqs. (45) and (46). The Γ_A^H versus m_t curves and the $\Gamma^H_{AH^+}$ versus m_t curves at several coexistent compositions of LC and LC•H$^+$ are shown in Fig. 7. The Γ_A^H values were larger than the corresponding $\Gamma^H_{AH^+}$ values at the coexistent composition. A greater quantity of LC than LC•H$^+$ existed in the adsorbed film. Since LC•H$^+$ molecules protonated from HCl become more hydrophilic, they are repulsive electrostatically among the polar head groups. The LC molecules without a proton can be transferred to a hydrophobic surface region more easily than the LC•H$^+$ molecules [13]. With decreasing X_2, the Γ_A^H values decreased, while the $\Gamma^H_{AH^+}$ values increased in proportion to the quantity of LC in the aqueous solution. Furthermore, we notice that the $\Gamma^H_{AH^+}$ values are negative in the higher X_2 range. The negative value of $\Gamma^H_{AH^+}$ means that the concentration of LC•H$^+$ in the surface layer, which is defined by two dividing planes, is lower than that in the bulk solution. The LC•H$^+$ molecules cannot transfer into the surface region owing to the preferential adsorption of the LC molecules and they are conversely excluded from the adsorbed film. Similar behavior was observed for a mixture of alkylammonium chlorides with a short hydrophobic chain [25].

The composition dependence of Γ_A^H and $\Gamma^H_{AH^+}$ at $m_t = 10$ mmol kg^{-1} is constructed from Fig. 7 and is shown in Fig. 8. We found from this figure that the partitioning of LC and LC•H$^+$ in the surface-adsorbed film can be separated into three composition regions: (1) small composition range ($0 \leq X_2 \leq 0.5$), slight partitioning of lower surface-active LC•H$^+$ because of no LC in the region; (2) intermediate composition range ($0.5 < X_2 <$ about 0.7), partitioning of both species, but preferential partitioning of LC compared with LC•H$^+$; and (3) large composition range (about $0.7 \leq X_2 \leq 1$), negative partitioning of LC•H$^+$ expelled from the adsorbed film formed by LC partitioned preferentially.

Because the physiological pH value in vivo has a value of about 7.4, we finally compare the dissociation equilibrium between LC and LC•H$^+$ in the aqueous solution with that in the adsorbed film at the pH. According to Eq. (47), more LC•H$^+$ molecules exist than LC ones in the aqueous solution as shown in Fig. 1B at pH 7.4. The composition dependence of the pH at $m_t = 10$ mmol kg^{-1}, which was obtained from Fig. 3, is drawn in the inset in Fig. 8. From the figure, the composition at pH 7.4 in this study corresponds to about 0.60. In the vicinity of this composition, the LC molecules preferentially partition into the adsorbed film. And conversely they exist in greater quantities than LC•H$^+$ in the adsorbed film as shown by the broken

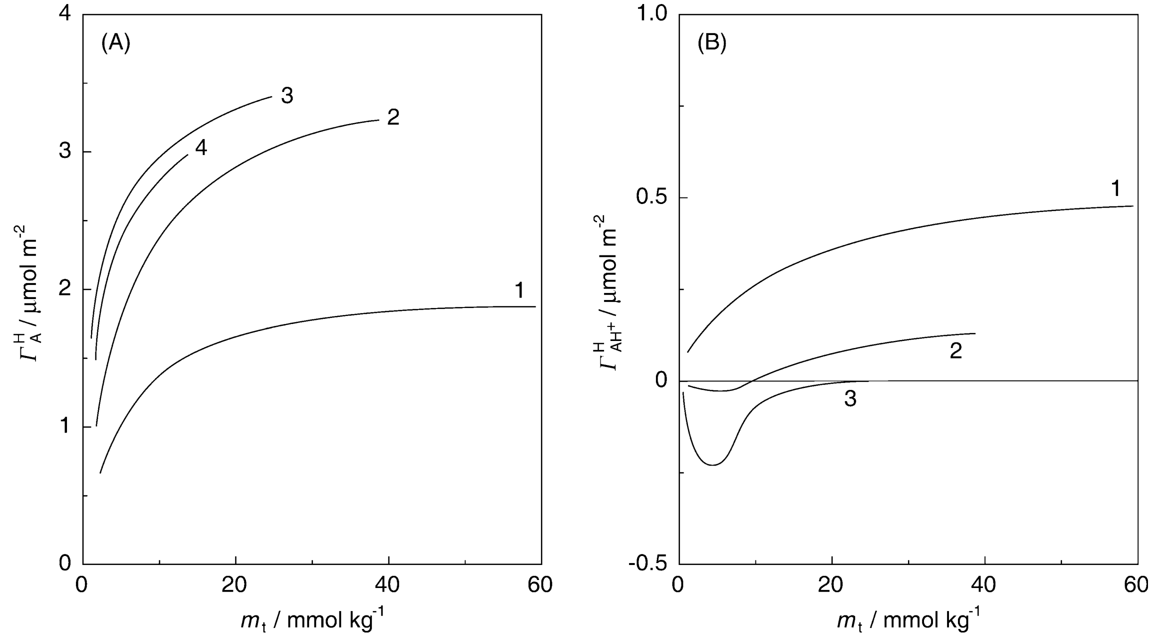

Fig. 7 **A** Surface density versus total molality curves of uncharged LC at constant composition: $X_2 = 0.600$ (*1*), 0.675 (*2*),0.750 (*3*), 1 (*4*). **B** Surface density versus total molality curves of charged LC at constant composition: $X_2 = 0.600$ (*1*), 0.675 (*2*), 0.750 (*3*)

line in Fig. 8. Therefore, we can say that uncharged local anesthetics preferentially partition into hydrophobic environments such as cell membranes compared with charged anesthetics even if the latter exist in greater quantities than the former in aqueous solution. The large difference in surface activities between uncharged and charged anesthetics in the coexistent solution is consistent with their partition coefficients obtained from our previous studies [26, 27, 28]. This supports the view that uncharged anesthetics play an important role in the molecular mechanism of local anesthesia [29, 30].

Conclusions

Tertiary amine local anesthetics exist as uncharged and charged forms in physiological solution. These forms interact in various modes with phospholipids and proteins in nerve membranes. The present study investigated the dissociation equilibrium between LC and LC•H$^+$ in a surface-adsorbed film from surface tension and pH measurements for a HCl–LC mixture. The ratio of both forms in the adsorbed film was evaluated from the thermodynamic equations derived. We found that a greater quantity of LC than LC•H$^+$ existed in the adsorbed film in the coexistent composition range, even if more LC•H$^+$ existed than LC in the aqueous solution. Considering the transfer of local anesthetic molecules into hydrophobic environments in cell membranes is an essential process for local anesthetic action, the ability of preferential partitioning for uncharged anesthetic molecules into hydrophobic environments is a remarkably important first step for the molecular mechanism of local anesthesia.

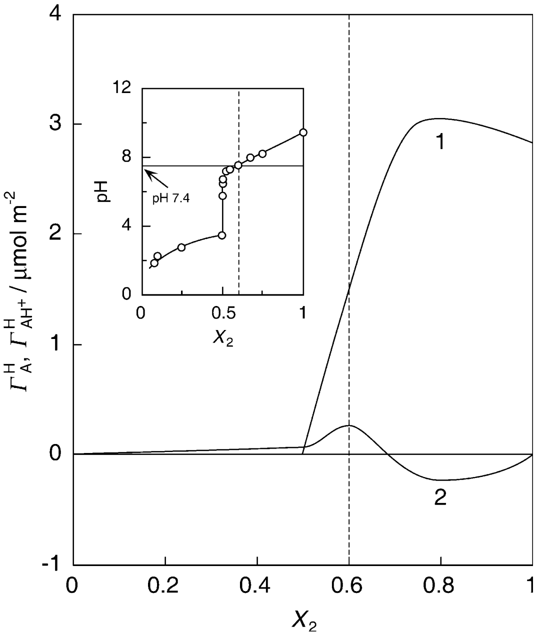

Fig. 8 Surface density of uncharged and charged LC versus composition curves at $m_t = 10$ mmol kg^{-1}: LC (*1*), LC•H$^+$ (*2*). The *inset* indicates the pH versus composition curve at $m_t = 10$ mmol kg^{-1}. The *broken line* corresponds to the composition (about 0.60) at pH 7.4

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