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Transient Membrane Potential in Response to a Bitter Substance in a Membrane Filter Impregnated with Phospholipid and 1-Octanol

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Transient and steady membrane potentials in response to a bitter substance have been measured in a membrane filter impregnated with phospholipid and 1-octanol. Quinine, which is one of the alkaloids, was used as the bitter substance. The internal and external solutions both contained 0.1 mol dm^{-3} KCl. A cation exchange liquid membrane was formed in a Teflon filter by impregnating it with a 1-octanol solution of dihexadecyl hydrogenphosphate (DCP). After the injection of the solution of quinine hydrochloride into the external solution, the membrane potential rapidly increased to reach a peak and gradually decreased to relax to a level of another steady potential. The time course curve of membrane potential was resolved in the early portion and the other portion after the peak to obtain the Donnan potential at the interface and the diffusion potential within the membrane at steady state, respectively. In comparison with the case of the response to HCl, even a small amount of aqueous quinine hydrochloride induced a great magnitude of change both in the Donnan potential and in the diffusion potential within the membrane. The amount of quinine transported across the membrane was measured by UV spectrophotometry. From the time dependence of the UV spectrum, we determined the time lag for the membrane diffusion to obtain the diffusion coefficient of quinine in the membrane. According to the theoretical expressions of the Donnan potential at the interface for the charged membrane and the intramembrane diffusion potential derived by solving the Nernst–Planck equations taking into account the Donnan equilibrium at the interface, these two constituents of the total membrane potential were estimated using the diffusion coefficient. By fitting the theoretical curves to the experimental results, large values of the partition coefficient and the equilibrium constant of the complex formation with DCP for monoprotonated quinine ion relative to potassium ion were obtained.

Although a human's sense of taste is diverse, bitter, salty, sour, sweet, and umami tastes are known as five basic tastes. In the initial process of the gustatory sensation, the adsorption of the substance to be tasted to the receptor membrane causes the generation of the membrane potential. The stimulation of a taste substance at the membrane surface initially induces a change of an analog type in the membrane potential. Then this chemical stimulation is transduced into the digital signals (impulses) in the taste nerves. For the receptor potential in the initial process, the generation mode depends on the types of taste substances.[1—3] While it is thought that a receptor protein in the taste receptor membrane plays a major part for sweet substances such as suger, umami substances such as monosodium glutamate and bitter substances of some type, it has been pointed out that there exists the process in which the lipid membrane itself functions as site of reception for the salty, sour and bitter tastes. Among the taste stimuli, bitter substances are most abundant. The most predominant of them are alkaloids which are known as electrolytes of hydrophobic ions. For these bitter substances, the threshold values of sensation are remarkably small compared with other taste substances. Because of the hydrophobic interaction with the lipid membrane, the changes in membrane potential in response to a bitter substance should differ from that in response to a sour or salty substance, which are both electrolytes of simple cations (proton or alkali metal ions). Through the studies concerning the electrochemical responses to various bitter substances, the importance of the change in the electrical potential at the membrane/solution interface as well as the strong interaction of these substances with the membrane has been suggested in the reconstituted bimolecular lipid membrane[4] and in the porous-filter membrane impregnated with di(9-octadecenyl) hydrogenphosphate.[5]

In charged membrane-aqueous electrolyte systems, the electrical potential difference (Donnan potential) is generated at the membrane/solution interface originating in the surface charge due to ionized groups of the membrane.[6,7] As a result, the total membrane potential involves the contribution of the Donnan potential in addition to that of the diffusion potential within the membrane. In previous papers, it has been demonstrated that the total membrane potential observed in the charged liquid membrane[8,9] as well as in the ion-exchange polymer membrane[10] can be divided into its two constituents on the basis of the measurement of the time dependence of the membrane potential. The response of the surface region of the membrane to the hydrophobic ion of the bitter substance is expected to be elucidated from the transient potential response.

In general, there are two factors determining the ion trans-

port across a membrane; distribution of permeable ions across the membrane/solution interface and mobility in the membrane. The properties of the interface have a strong effect on the distribution of ions, so that the surface concentration of ions is governed by this process along with the interactions with ionized groups on the membrane. The liquid membrane formed with a carrier substance and 1-octanol has been chosen as a membrane in which these physicochemical properties of the interface can well be specified.[11,12] The reason why 1-octanol has been used as solvating medium is mentioned by many workers.[13,14] On the basis of the systematic investigations of the partition coefficients in 1-octanol/water systems, the significance of close correlation of these properties for many organic compounds with their biological activities, e.g., the anesthetic action, has been deduced.

In this study, quinine, which is one of the alkaloids, has been chosen as a bitter substance. The changes in membrane potential in response to quinine in the monolayer coated liquid membrane with a phospholipid have been investigated. The characteristics of the changes in membrane potential have been discussed.

Experimental

1. Materials. A supported liquid membrane was prepared by soaking a membrane filter in 1-octanol with 5×10^{-4} mol dm^{-3} dihexadecyl hydrogenphosphate (DCP) (Aldrich, Milwaukee) under a reduced pressure. We used a membrane filter made from polytetrafluoroethylene with an average pore size of 1.0 μm, and the thickness of 200 μm or 100 μm (Fluoropore FP-100, Sumitomo Electric Co., Osaka). Prior to preparation of the membrane forming solution, water of 3.0% by weight was dissolved in the 1-octanol (Sigma, St Louis). A KCl solution used as an electrolyte solution was equilibrated with the membrane-forming solution before measurement. An aqueous solution of quinine hydrochloride dihydrate (Fluka, Switzerland) was prepared. These reagents used in this study were the highest grade and were used without further purification. Water was purified by double distillation, and once from alkaline solution of $KMnO_4$.

2. Measurements. Membrane Potential. A schematic diagram of the assembled system for the measurement of membrane potential is shown in Fig. 1. The supported liquid membrane was mounted horizontally in the cell for the measurement. The area of membrane surface adjacent to the electrolyte solution (diffusion area) was 0.20 cm^2. Initially, both external and internal solutions contained 0.1 mol dm^{-3} KCl so that there was no ion gradient across the membrane. An amount of aqueous solution of quinine hydrochloride was injected out of the tip of an auto piston burette (Model APB-410, Kyoto Electronics, Kyoto) equipped with an autoinjection regulator (Model AIN-410, Kyoto Electronics), into the external solution. This additive solution also contained 0.1 mol dm^{-3} KCl to keep the concentration of K^+ ion in the external solution constant. The membrane potential was measured by a pair of Ag–AgCl reference electrodes with liquid junction (Model K-801, Radiometer, Copenhagen) connected to an electrometer (Model 614, Keithley, Cleveland, Ohio). The injection speed was controlled at 1 cm^3 s^{-1} so that the membrane potential started to change after the injection was completed. The concentration of quinine in the external solution varied with changes in the injection amount of the solution of quinine hydrochloride. The effect of stirring speed on the membrane potential was examined to find out that, at the rate of 550—650 rpm, the time course curve was not affected by reducing the speed at all. Each solution was stirred at 650 rpm by a magnetic stirrer. Throughout the measurement, the temperature of the membrane system was maintained at 25.0 °C by circulating thermostatted water.

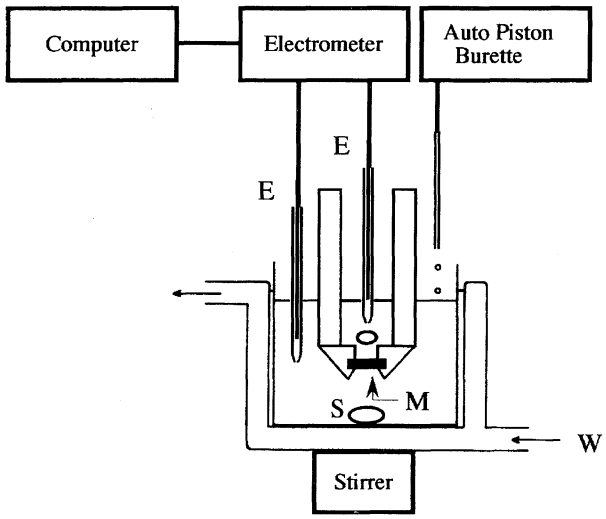

Fig. 1. Schematic diagram of the assembled system for the measurement of membrane potential. Supported liquid membrane (M) was mounted horizontally in the cell for the measurement. The membrane potential was measured by a pair of Ag–AgCl reference electrodes (E) connected to an electrometer. Aqueous solution of quinine hydrochloride was injected into the external solution by an auto piston burette. Each solution was stirred with a magnetic spin bar (S). The temperature was maintained at 25.0 °C by circulating thermostatted water (W) through the water jacket.

pH of Aqueous Solution of Quinine Hydrochloride. Quinine has two N atoms in the nuclei of quinoline and quinuclidine. The values of pK_b's of quinine are 8.9 and 4.3.[15] The values of pK_b of quinoline and that of quinuclidine are 9.5[16] and 3.4,[17] respectively. The change in pH of the external KCl solution (0.1 mol dm^{-3}) in response to the injection of quinine hydrochloride was measured by a glass electrode connected to a pH meter (Model F-80, Horiba, Kyoto). The concentration of quinine hydrochloride after the injection ranged from 1×10^{-4} mol dm^{-3} to 3×10^{-4} mol dm^{-3}. Initially, pH of KCl solution was about 5.6. With injection of quinine, pH of the solution slightly increased to a pH in the range of 5.6—5.8. The results suggest that quinine exists almost perfectly as monoprotonated quinine ion in aqueous solution of quinine hydrochloride.

Amount of Quinine Transported across the Membrane. The amount of quinine transported across the membrane was determined by measuring the absorption spectra of quinine of the internal solution to obtain the diffusion coefficient of quinine. Figure 2 shows the schematic diagram of the assembled system for the measurement of the amount transported across the membrane. A probe with a light-path length of 1 cm through the solution was connected to a fiber optic spectrophotometer (Model S2000, Ocean Optics, Dunedin, Florida) through an optical fiber. The probe was immersed in the internal solution to measure the absorption spectra. The other experimental conditions were the same as for the measurement of membrane potential.

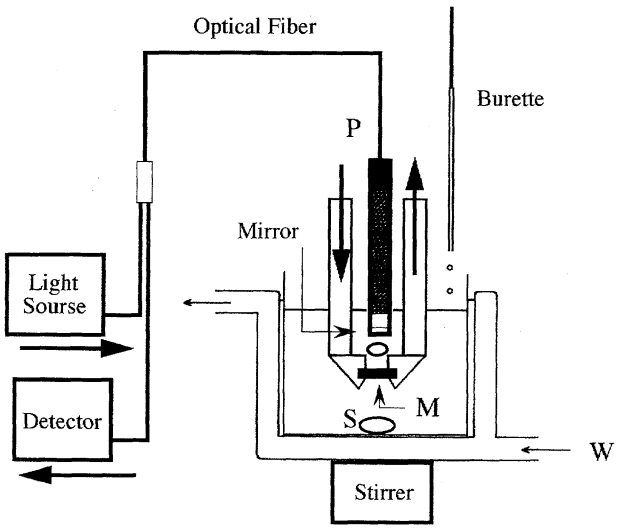

Fig. 2. Schematic diagram of the assembled system for the measurement of amount of quinine transported across the membrane. Time dependence of the amount of quinine in the internal solution was obtained through the measurement of absorption spectrum using an immersed probe (P) connected to a UV spectrophotometer. Arrows (→) show the path of ultraviolet radiation. Supported liquid membrane, magnetic spin bar, and thermostatted water are designated by M, S, and W, respectively.

Theoretical

Consider the membrane system with a planer liquid membrane supported on a filter. The membrane separates the external solution (solution I) and the internal solution (solution II), both containing 0.1 mol dm^{-3} KCl. An amount of aqueous quinine hydrochloride was added in the external solution. We assume that co-ions (Cl^- ions) are perfectly excluded from the negatively charged liquid membrane and that the lipid molecules are allowed to exist only in the membrane.

The flux of ionic species i, J_i, is expressed by the Nernst–Planck equation as

$$J_i = c_i u_i \left(-\frac{d\tilde{\mu}_i}{dx}\right) \qquad (i = K^+, QH^+, L^-) \tag{1}$$

where c and u denote the concentration and the mobility, respectively. QH^+ and L^- designate protonated quinine ion and free lipid ion having one negative charge, respectively. The electrochemical potential of ionic species i is given by Eq. 2:

$$\tilde{\mu}_i = \mu_i^\circ + RT\ln a_i + Z_i F\phi, \tag{2}$$

where μ° is the standard chemical potential; a is the activity; Z is the charge number; and ϕ is the electrical potential. R, T, and F are gas constant, temperature, and Faraday constant, respectively. The condition of zero electric current across the membrane can be written as

$$I = F(J_K + J_{QH} - J_L) = 0. \tag{3}$$

Here, consider the membrane system where no ion transport due to the osmotic pressure is observed. Substituting Eqs. 2 and 3 into Eq. 1 and integrating across the membrane on the assumption of the constant electric-field,[18] one can obtain the diffusion potential, $\Delta\phi$, under the condition of the steady state as

$$\Delta\phi = \phi^{in} - \phi^{ex} = \frac{RT}{F}\ln\frac{Y_-}{Y_+}, \tag{4}$$
$$Y_- = u_K c_K^{ex} + u_{QH} c_{QH}^{ex} + u_L c_L^{in},$$
$$Y_+ = u_K c_K^{in} + u_{QH} c_{QH}^{in} + u_L c_L^{ex},$$

where superscripts ex and in designate the surface on the membrane of the external solution (solution I) side and that on the internal solution (solution II) side, respectively. The mobility of ionic species is assumed to be constant within the membrane. The translocation of ionic species through the membrane is slow compared with the rate at which the ionic species flows across the interface, so that the Donnan equilibrium can be reached at the interface immediately after the change in composition of the external solution. The Donnan equilibrium at the interface of the external solution and the membrane is expressed as

$$\mu_i^{oI} + RT\ln a_i^I + Z_i F\phi^I = \mu_i^{oex} + RT\ln c_i^{ex} + Z_i F\phi^{ex}, \tag{5}$$

where superscript I refers to solution I. According to Eq. 5, the concentration of ionic species i at the membrane surface on the external solution side, c_i^{ex}, can be expressed in terms of the Donnan potential ($U^I = \phi^{ex} - \phi^I$) and the activity of ionic species i in the aqueous solution as

$$c_i^{ex} = a_i^I b_i \exp(-Z_i F U^I/RT). \tag{6}$$

Taking account of the competitive reaction between two kinds of counter ions (K^+ and QH^+) with ionized groups of the lipids in the membrane, we obtain the Donnan potential at the interface on the external solution side, U^I, by Eq. 7:[19]

$$U^I = \phi^{ex} - \phi^I = -\frac{RT}{F}\ln\left[-\frac{1}{2A} + \left\{\left(\frac{1}{2A}\right)^2 + \frac{c_L^T}{BA}\right\}^{1/2}\right], \tag{7}$$
$$A = b_K K_K a_K^I + b_{QH} K_{QH} a_{QH}^I,$$
$$B = b_K a_K^I + b_{QH} a_{QH}^I,$$

where c_L^T is the total concentration of ionizable groups of the lipids within the membrane; b and K represent the partition coefficient and equilibrium constant of complex formation reaction between free lipid ion L^- and ionic species i given by Eqs. 8 and 9, respectively.

$$b_i = \exp\left\{-\frac{(\mu_i^{oex} - \mu_i^{oI})}{RT}\right\}, \tag{8}$$

$$K_i = \frac{c_{ci}}{c_i c_L}. \tag{9}$$

The subscript ci refers to the complex between the lipid ion and ionic species i. It has been assumed here that the electroneutrality condition at the surface on the membrane holds and that the activity coefficients for all chemical species in the membrane are equal to unity. The Donnan potential at

the interface on the side of the internal solution, U^{II}, can be derived in a similar manner.

Hence the total membrane potential, U_m, can be expressed by the sum of the diffusion potential within the membrane and the Donnan potential as

$$\begin{aligned} U_m &= \phi^{II} - \phi^{I} \\ &= (\phi^{II} - \phi^{in}) + (\phi^{in} - \phi^{ex}) + (\phi^{ex} - \phi^{I}) \\ &= -U^{II} + \Delta\phi + U^{I}. \end{aligned} \tag{10}$$

Results and Discussion

Figures 3 and 4 show the time dependence of membrane potential in response to quinine. As seen from these figures, the membrane potential rapidly increased to reach a peak after the injection of the solution of quinine hydrochloride and gradually decreased to relax to a level of another steady membrane potential. On the basis of the theory of the membrane potential for the charged membrane described in the previous section, the early fast step and the slow process after the peak can be regarded as the change in the Donnan potential and that in the diffusion potential within the membrane, respectively. Therefore, the time dependence of membrane potential was divided into the early portion and the other portion after the peak. Since the flow of ionic species in the membrane interior is much slower than the flow across the membrane/solution interface, it is thought that the diffusion potential does not start to change until the Donnan potential reaches the peak. The Donnan potential changed in the positive direction in response to the injection of the solution of quinine hydrochloride. On the other hand, the diffusion potential within the membrane changed in the negative direction, suggesting that the mobility factor of QH^+ ion is much

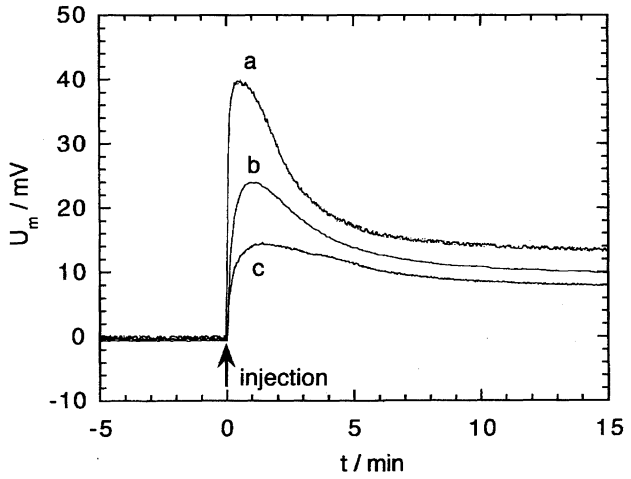

Fig. 3. Time dependence of membrane potential in response to quinine. The arrow indicates the time when the injection of the aqueous solution of quinine hydrochloride into the external solution was carried out. Concentration of quinine in the external solution varied with changes in the injected amount of the solution of quinine hydrochloride: (a) 6×10^{-5} mol dm^{-3}; (b) 3×10^{-5} mol dm^{-3}; (c) 2×10^{-5} mol dm^{-3}. The membrane thickness was 100 μm.

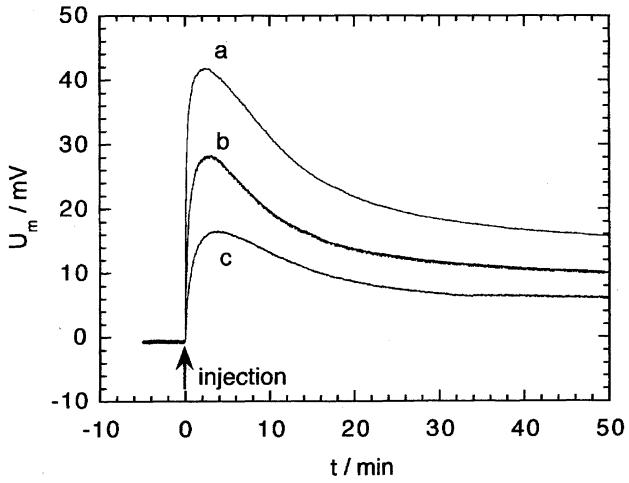

Fig. 4. Time dependence of membrane potential in response to quinine. The membrane thickness was 200 μm. Other experimental conditions were the same as for the series in Fig. 3. Concentration of quinine in the external solution after the injection: (a) 6×10^{-5} mol dm^{-3}; (b) 3×10^{-5} mol dm^{-3}; (c) 2×10^{-5} mol dm^{-3}.

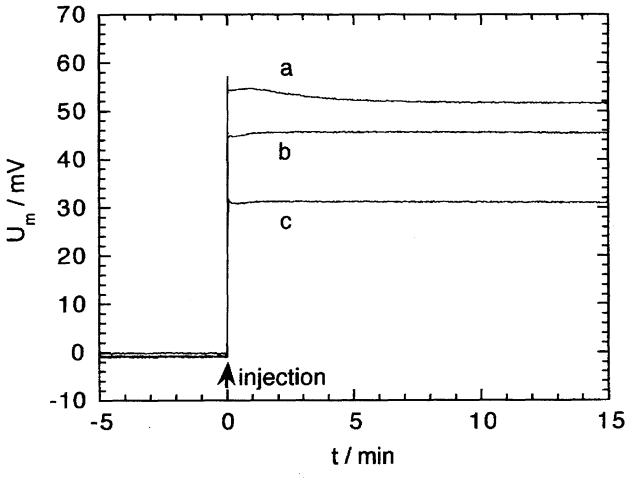

Fig. 5. Time dependence of membrane potential in response to hydrochloric acid. Concentration of HCl in the external solution after the injection: (a) 5×10^{-3} mol dm^{-3}; (b) 3×10^{-3} mol dm^{-3}; (c) 1×10^{-3} mol dm^{-3}. The membrane thickness was 100 μm.

smaller than that of K^+ ion, because the contribution of Cl^- ion is negligible. As seen from the comparison between Fig. 3 and Fig. 4, the effect of the membrane thickness on the time dependence of the membrane potential was observed. The rates of relaxation of the membrane potential after the peak were dependent on the thickness of the membrane the ions have to traverse. This suggests that this slow process is due to the diffusion of ionic species within the membrane.

Figure 5 shows the time dependence for the hydrochloric acid which elicits sour taste. As shown in this figure, the membrane potential rapidly increased immediately after the injection of HCl to reach the peak and then decreased slightly. It was found that, in comparison with the case of HCl, even a small amount of quinine hydrochloride induced a great

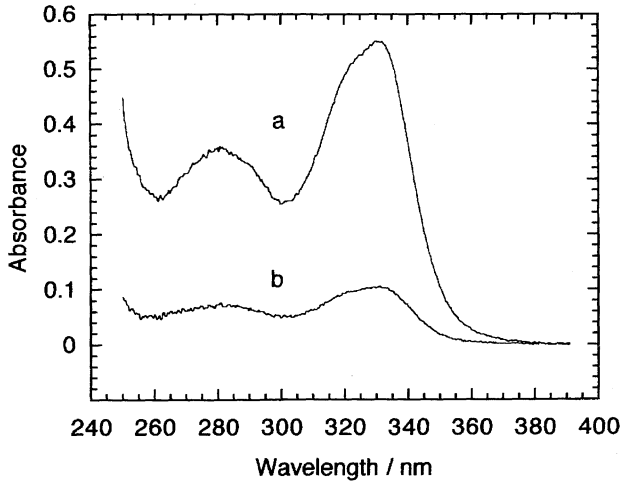

Fig. 6. Absorption spectra of quinine hydrochloride in aqueous solution of 0.1 mol dm^{-3} KCl. Concentration of quinine hydrochloride: (a) 1×10^{-4} mol dm^{-3}; (b) 2×10^{-5} mol dm^{-3}.

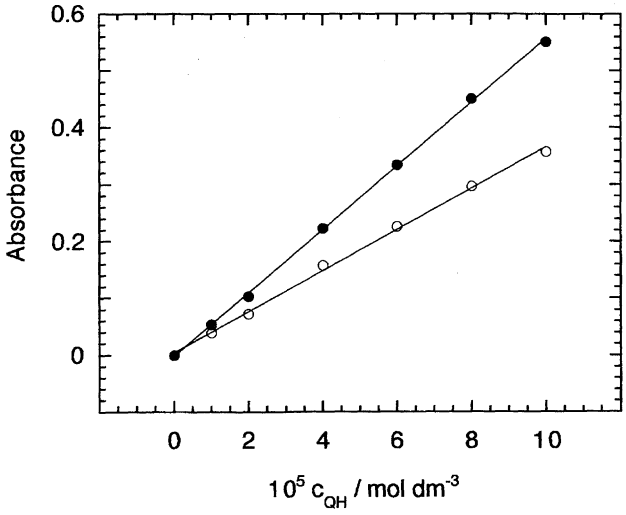

Fig. 7. Calibration curves. Plots of absorbance of quinine hydrochloride in aqueous solution of 0.1 mol dm^{-3} KCl at 330 nm (●) and 280 nm (○) versus concentration of quinine hydrochloride.

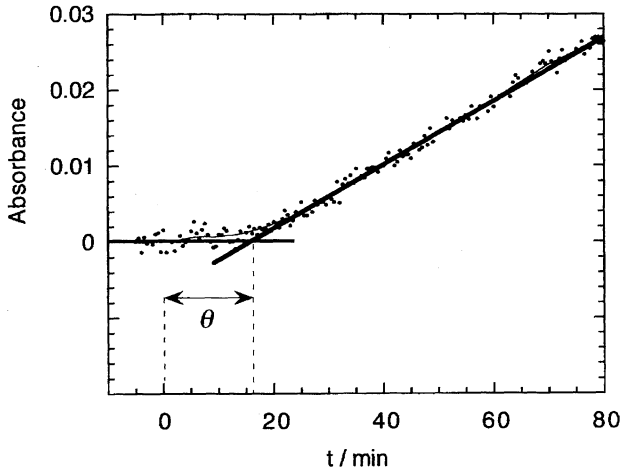

Fig. 8. Time dependence of absorbance. Absorbance changes in the internal solution at 330 nm obtained from the absorption spectrum mean the total quantity of quinine transported across the membrane. The small amount of aqueous solution of quinine hydrochloride was injected into the external solution at the time zero ($t = 0$). Concentration of quinine in the external solution after the injection was 4×10^{-4} mol dm^{-3}. The membrane thickness was 200 μm. Time lag, θ, is shown by the point where the straight line of steady state and the time-axis intersect.

magnitude of change both in the Donnan potential and in the diffusion potential within the membrane.

By UV spectrophotometry, we measured the amount of quinine transported across the liquid membrane in the same membrane system as for the potential measurement. In order to obtain the diffusion coefficient in 1-octanol for quinine, we used the membrane filter impregnated with membrane solvent (1-octanol containing 3.0% water) without lipids. First, the absorption spectra of quinine hydrochloride in aqueous solution of 0.1 mol dm^{-3} KCl were examined. As shown in Fig. 6, there existed two absorption maxima of 280 and 330 nm in the range of wavelength of UV measured. The calibration curve for the absorbance at each absorption maximum is shown in Fig. 7. It is seen from this figure that plotting of the absorbance versus the concentration results in a straight line passing through the origin. These maxima and the molar absorbtivities satisfactorily agree with the values reported in the literature.[20]

Choosing 330 nm as the wavelength, the total quantities of quinine transported across the membrane were determined. As shown in Fig. 8, the absorbance increased with an induction period after the injection of quinine hydrochloride and then reached a line at the steady state. We have measured the flux across the uncharged liquid membrane without ionic carriers to determine the diffusion coefficient of quinine in the membrane solvent. The total flux of quinine can be assumed to be given by the sum of the flux of monoprotonated quinine ion and that of quinine hydrochloride having no electric charge because the fraction of quinine of neutral form in aqueous phase can be neglected.

The diffusion equation at the non-steady state can be written by the material balance equations for quinine as

$$\frac{\partial c_{QH}}{\partial t} = -\left(\frac{\partial J_{QH}}{\partial x}\right) - k_1 c_{QH} c_{Cl} + k_2 c_{QHCl}, \quad (11)$$

$$\frac{\partial c_{QHCl}}{\partial t} = -\left(\frac{\partial J_{QHCl}}{\partial x}\right) - k_2 c_{QHCl} + k_1 c_{QH} c_{Cl}, \quad (12)$$

where k_1 and k_2 are the rate constants of the forward and the backward reactions for the formation of the complex between quinine cation, QH^+, and Cl^- ion. Subscript QHCl refers to the complex. From Eqs. 11 and 12, we obtain the balance equation for the total amount of quinine in the membrane as

$$\frac{\partial(c_{QH} + c_{QHCl})}{\partial t} = -\left(\frac{\partial(J_{QH} + J_{QHCl})}{\partial x}\right). \quad (13)$$

The term of J_{QH} can be replaced with the salt flux, $J_{\pm}$, because $J_{QH} = J_{Cl} = J_{\pm}$. We have assumed that the concentration of quinine of neutral form within the membrane

is negligibly low compared with that of protonated quinine ion. Therefore,

$$\frac{\partial(c_{QH}+c_{QHCl})}{\partial t} = -\left(\frac{\partial(J_{\pm}+J_{QHCl})}{\partial x}\right). \quad (14)$$

According to Fick's first law, we obtain

$$J_{\pm} = -D_{\pm}\left(\frac{\partial c_{\pm}}{\partial x}\right) \quad (15)$$

$$J_{QHCl} = -D_{QHCl}\left(\frac{\partial c_{QHCl}}{\partial x}\right) \quad (16)$$

where $c_{\pm} = (c_{QH}c_{Cl})^{1/2}$. By the Nernst–Hartley relation,[21] $D_{\pm}$ is expressed in terms of the mobilities of quinine ion, u_+ ($u_{QH} \equiv u_+$), and chloride ion, u_- ($u_{Cl} \equiv u_-$), as

$$D_{\pm} = \frac{2RTu_+u_-}{u_+ + u_-}\left(1 + \frac{d\ln y_{\pm}}{d\ln c_{\pm}}\right) \quad (17)$$

where $y_{\pm}$ denotes the mean activity coefficient. Provided that

$$D_{\pm} = D_{QHCl} \equiv D, \quad (18)$$

Eq. 14 yields an equation of the same form as for the case of single species:[22]

$$\frac{\partial(c_{QH}+c_{QHCl})}{\partial t} = D\left(\frac{\partial^2(c_{QH}+c_{QHCl})}{\partial x^2}\right). \quad (19)$$

Therefore, by solving Eq. 19 under the initial condition of zero concentration over the whole membrane system, the expression of the time lag θ for the transport across the membrane is obtained as

$$\theta = \frac{l^2}{6D}, \quad (20)$$

where l denotes the membrane thickness.

Time lag, θ, was obtained from the point where the straight line of steady state and the time-axis intersect, as demonstrated in Fig. 8. For the membrane with a thickness of 200 μm, θ was 16 min. The diffusion coefficient of QH^+ ion in 1-octanol, D_{QH}, was calculated according to Eq. 20 to obtain the value of 6.8×10^{-12} m^2 s^{-1}. The value of D_{QH} calculated from θ for the membrane of 100 μm thickness nearly agreed with that for 200 μm. The value of diffusion coefficient obtained here includes the porosity of the membrane filter. In comparison with the transient membrane potential shown in Fig. 4, the time for the diffusion process to attain the steady state satisfactorily agreed with the time to attain the steady potential for the relaxation process of the membrane potential. This supports the conclusion that the slow process after the peak in the membrane potential change stems from the diffusion of permeable ionic species within the membrane.

The Donnan potential and the diffusion potential within the membrane were theoretically calculated according to Eqs. 4 and 7 by using the value of D_{QH} obtained from the time lag measurement. The values of diffusion coefficient of K^+ ion in 1-octanol, D_K, and that of partition coefficient of K^+ ion from solution to membrane at the interface, b_K, in the references were used. The value of D_K was determined from the limiting molar conductivity[11] according to the Nernst–Einstein equation to be 4.5×10^{-11} m^2 s^{-1}. The activity coefficients of ions in aqueous solutions were calculated according to the Debye–Hükel equation. Figure 9 also shows the calculated membrane potentials together with the experimental values. The ratio of equilibrium constants of formation reaction of the complex, K_{QH}/K_K, and that of partition coefficient, b_{QH}/b_K, obtained from the best fitting of the theoretical values to the experimental results were 3.3 and 3.0×10^3, respectively. The value of b_K in 1-octanol/water system is 1.6×10^{-4} [12] provided that $b_K = b_{Cl}$. From this value and the best fitted value of b_{QH}/b_K, b_{QH} can be estimated to be 0.48. The order of magnitude of b_{QH} is smaller than that of the partition coefficient of the uncharged quinine of free base: b_Q ($b_Q = 54$[13]). The results suggest that the distribution of QH^+ ion can be explained by the order nearly equal to that of "mean partition coefficient", $b_{\pm}$, between b_Q and b_{Cl}, i.e., $b_{\pm} = (b_Q b_{Cl})^{1/2}$. Moreover, we considered the effect of the mobility of lipid ion as an ionic carrier. The membrane potentials have been calculated by putting the mobility of the free lipid ion to be equal to that of QH^+ ion. As seen from Fig. 9, the effect of the mobility of lipid on the diffusion potential gradually increases in the higher concentration range. This change in diffusion potential within the membrane causes an increase of the total membrane potential.

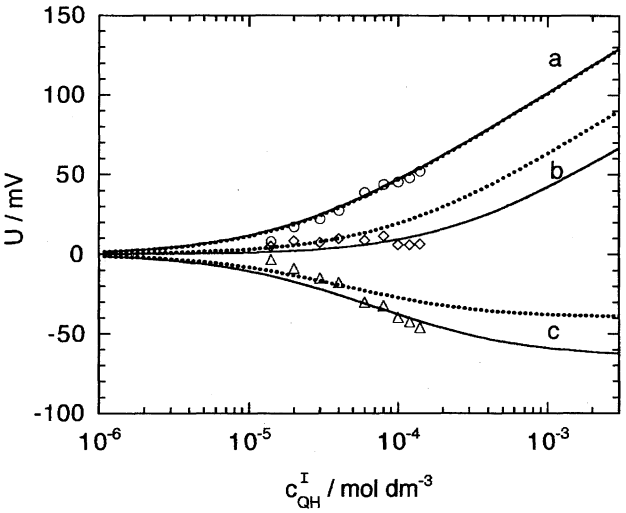

Fig. 9. Plots of Donnan potential and diffusion potential within the membrane as a function of concentration of quinine in the external solution. Time dependence of membrane potential shown in Fig. 3 has been resolved into the early fast step and the slow process after the peak to obtain the Donnan potential (○) and the diffusion potential within the membrane (△), respectively. Together with these two constituents, the total membrane potential at steady state (◇) is shown. The solid lines indicate theoretically calculated values in the case where the mobility of DCP can be neglected: (a) the Donnan potential; (b) the steady membrane potential; (c) the diffusion potential within the membrane. The dotted lines indicate theoretically calculated values in a case where the mobility of DCP is assumed to be equal to that of quinine ion.

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