

magnetic axes of the product diyl. It is possible that the symmetry of the photoexcited diazene is conferred upon the singlet diyl. The dinitrogen is certainly produced in its ground electronic state $^1\Sigma_g^+$. (The first electronic excitation of N_2 to $^3\Sigma_u^+$ is at 50 200 cm^{-1} .) Approximate conservation of symmetry suggests that the polar x symmetry is conferred on the diyl fragment of the photodis-

sociation.

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Chemoreception by an Excitable Liquid Membrane: Characteristic Effects of Alcohols on the Frequency of Electrical Oscillation

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Abstract: Studies were made on oscillations across a liquid membrane consisting of an oil layer, nitrobenzene containing picric acid, between two aqueous layers: that on the left containing 5 mM CTAB plus an alcohol at various concentrations and that on the right containing 0.1 M sucrose. This system showed sustained rhythmic oscillations of electrical potential of 200–400 mV with an interval on the order of 1 min. The frequency of oscillations increased with increase in the concentration of the alcohol. The critical concentration of alcohols needed to induce oscillations decreased with an increase in their hydrophobicity. The oscillations can be explained by a mechanism of repetitive formation and abrupt destruction of a monolayer structure of CTA⁺ on the interface between the organic and aqueous phases. The response to alcohols in the liquid membrane apparently resembled that of biological chemoreceptive membranes. The possibility was suggested of developing a new type of chemical sensor with the ability to distinguish various chemical substances from the patterns of electrical oscillation that they induced.

One of the most interesting phenomena in biological systems is excitability. There is much literature on electrical phenomena accompanying electrical excitation in biological membranes, but despite extensive studies on biooscillations, the physicochemical mechanisms of these phenomena are not yet clear. For an understanding of the mechanism of biological excitation and/or oscillation, various types of artificial membranes with excitability have been investigated.^{1–6} Most of the artificial membranes examined were excitable under an external force, such as pressure,^{1,2} voltage,^{3,5} or electrical current.^{3,4,6} In excitable biomembranes, it is well established that the difference in the compositions of electrolytes, especially potassium and sodium ions, across the membranes is important. Investigations on "self-excitable" artificial membranes are thus important in understanding the mechanism of excitation and/or oscillation in biological systems. However, there are very few reports on this phenomenon in artificial membranes. Kobatake⁴ found that porous membranes doped with dioleoylphosphate, DOPH-Millipore membranes, showed spontaneous firing of an electrical potential when placed between solutions of different KCl concentrations. Pant and Rosenberg⁷ observed that a lipid bilayer membrane separating bathing solution compartments of potassium ferricyanide and potassium iodide could be set into sustained electrical oscillations when the pH's of the two compartments were adjusted to 5 and 10, respectively. von Klitzing et al.⁸ reported that voltage spikes were generated

in a lipid bilayer membrane (black membrane) between aqueous solutions of different concentrations with KCl or KH_2PO_4 , though they observed no dependence of these spikes on the electrolyte concentration.

Recently, we⁹ found that electrochemical oscillations occur spontaneously even in a simple two-phase system consisting of an organic solution of picric acid in nitropropane and an aqueous solution of cetyltrimethylammonium bromide (CTAB). We also studied¹⁰ an artificial liquid membrane consisting of an oil layer, nitrobenzene containing picric acid, imposed between two aqueous phases, one containing 5 mM CTAB and 5% ethanol. We found that this system showed rhythmic and sustained oscillations of electrical potential within the range of 150–300 mV with an interval of 2–3 min. No oscillations were observed in the absence of ethanol. As an extension of these studies, we investigated the effect of alcohols on the oscillations in the liquid membrane.

The present paper reports the results of these experiments, indicating that the frequency of the oscillations depends on the concentration of alcohols, and that the threshold concentration needed to induce electrical oscillations decreases with an increase in the "hydrophobicity" of the alcohols.

Experimental Section

Experiments were performed in an apparatus with a U-shaped glass tube (12-mm inner diameter). The apparatus is shown schematically in Figure 1. A solution (4 mL) of 1.5 mM picric acid in nitrobenzene was placed in the base of the U cell. Aqueous solutions (10 mL each) were introduced simultaneously into the arms of the U cell above the organic phase without stirring. All measurements were carried out at 25 °C. The

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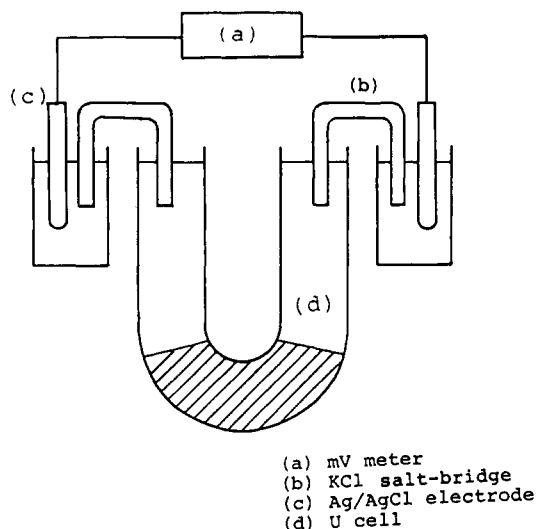


Figure 1. Diagram of the experimental apparatus.

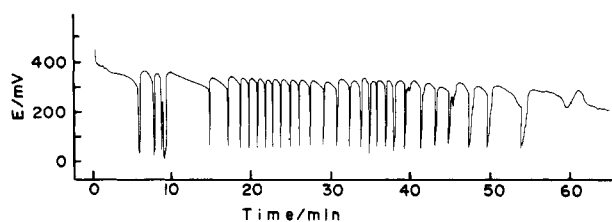


Figure 2. Oscillations of electrical potential between the two aqueous phases. A solution (4 mL) of 1.5 mM picric acid in nitrobenzene was placed in the base of the U cell. Aqueous solutions (10 mL) were introduced simultaneously into the arms of the U cell on each side of the organic phase. The aqueous solution in the left arm contained 5 mM CTAB and 1.5 M ethyl alcohol and that in the right contained 0.1 M sucrose. In the record of the electrical potential, an upward change denotes an increase in the positive charge in the right aqueous phase.

voltage across the liquid membrane was measured with a Hitachi-Horiba F-7 pH/mV meter connected by two salt bridges to two Ag/AgCl electrodes. All reagents were commercial products of analytical grade. Before use, nitrobenzene was purified by distillation and picric acid was dried in vacuo.

Throughout the experiments, the concentrations of CTAB and picric acid were fixed at 5 and 1.5 mM, respectively, which resulted in oscillations of nearly constant amplitude and frequency during the oscillatory period.¹¹

Results

Oscillations across the Liquid Membrane. Figure 2 shows the oscillations of voltage across a liquid membrane consisting of an oil layer, nitrobenzene containing 1.5 mM picric acid, between two aqueous phases, 5 mM CTAB plus 1.5 M ethyl alcohol on the left and 0.1 M sucrose on the right. The oscillations started 7 min after the organic phase came in contact with the two aqueous solutions and continued for about 1 h. The amplitude of the oscillations was between 250 and 300 mV. These oscillations were seen even when ethyl alcohol was added later. No oscillations were observed in the absence of ethyl alcohol or CTAB. The decrease in amplitude of the oscillations with time was found to be less in the presence of sucrose. This can be seen by comparison of Figure 2 with Figure 1 in our previous report,¹⁰ which showed that the amplitude of oscillations decreased gradually in the absence of sucrose.

Figure 3 shows the rhythmic oscillations observed with different concentrations of ethyl alcohol. With an increase in the con-

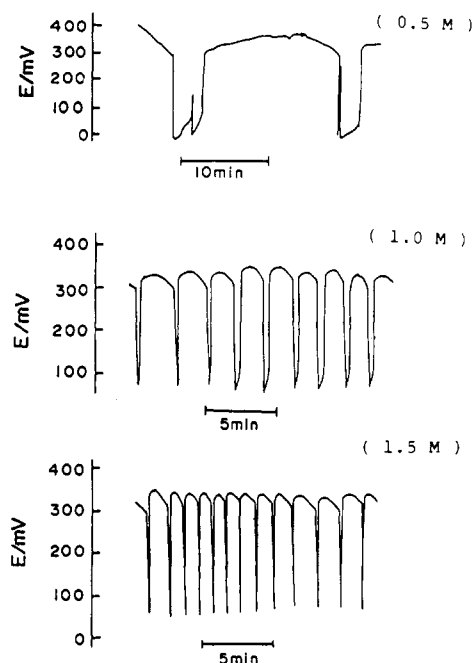


Figure 3. Oscillations of electrical potential with different concentrations of ethyl alcohol in the left aqueous phase. Other experimental conditions were the same as for Figure 2.

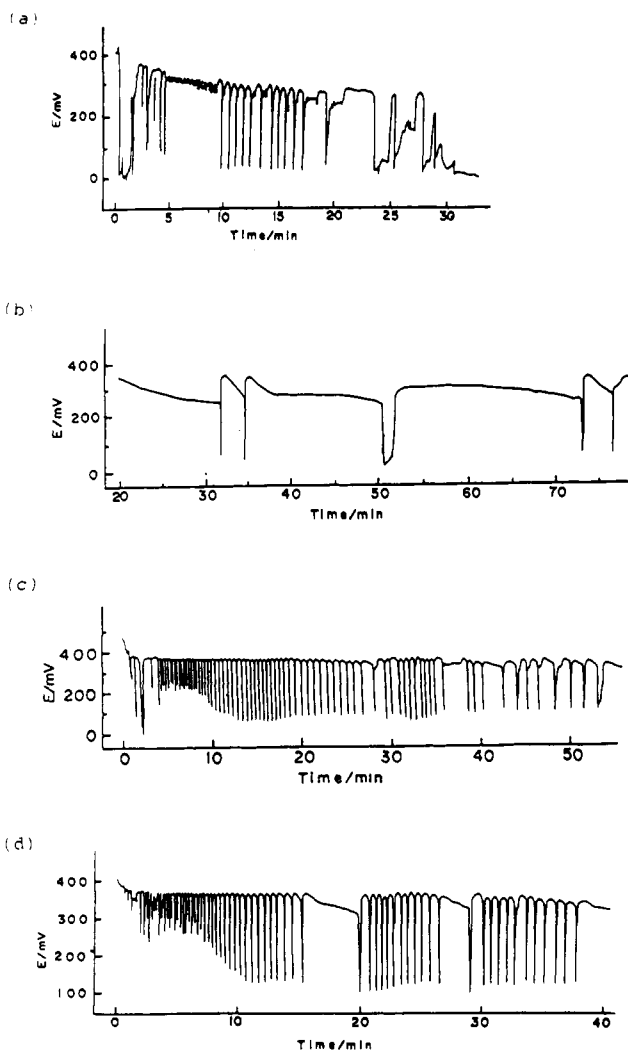


Figure 4. Oscillations of electrical potential with (a) 0.5 M *n*-propyl alcohol, (b) 0.5 M isopropyl alcohol, (c) 0.5 M *n*-butyl alcohol, and (d) 0.25 M *n*-pentyl alcohol in the left aqueous phase. Other experimental conditions were the same as for Figure 2.

(11) When the concentration of CTAB was decreased the oscillatory period tended to shorten and no oscillations occurred at CTAB concentrations of below 2.5 mM. When the concentration was increased above 5 mM, the oscillatory period became longer. When concentration of picric acid was decreased the oscillatory period became shorter and with an increase in the concentration above 1.5 mM the frequency tended to increase and the amplitude to decrease.

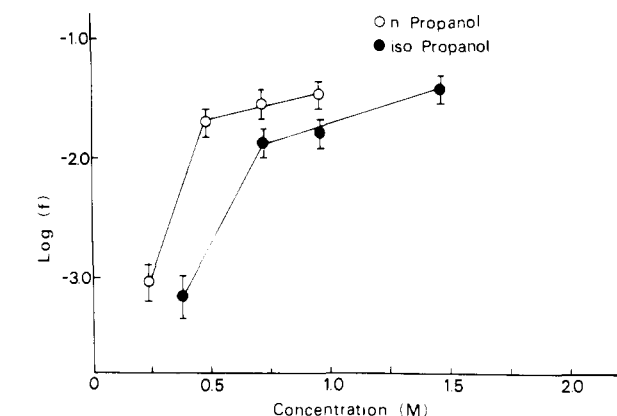
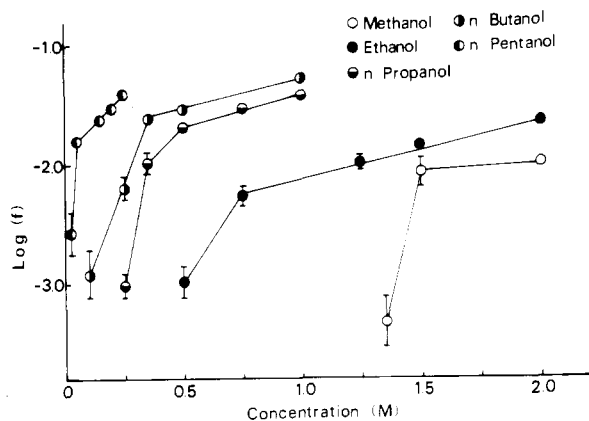


Figure 5. Concentration dependence of the frequency (f) of oscillations with methyl alcohol, ethyl alcohol, n -propyl alcohol, n -butyl alcohol, n -pentyl alcohol, and isopropyl alcohol. At each concentration, 3–5 experimental runs were carried out. Vertical bars represent 95% confidence intervals of the means.

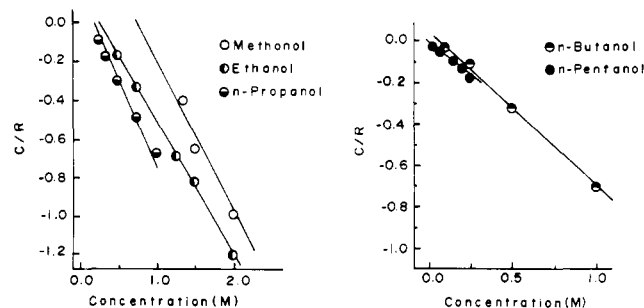


Figure 6. Relationship between C/R and C . C is the concentration of an alcohol, and R is the logarithm of the frequency of oscillations— $R = \log f$.

centration of ethyl alcohol, the frequency of oscillations increased. The amplitude of the oscillations was scarcely affected by the concentration of ethyl alcohol.

Oscillations could also be generated when ethyl alcohol was replaced by other aliphatic alcohols. Figure 4 shows the oscillations of electrical potential with 0.5 M n -propyl alcohol (Figure 4a), 0.5 M isopropyl alcohol (Figure 4b), 0.5 M n -butyl alcohol (Figure 4c), and 0.25 M n -pentyl alcohol (Figure 4d). It is noteworthy that the amplitudes of these oscillations were nearly the same, but their frequencies were very different.

Dependence of Frequency on Concentration of Alcohols. Figure 5 shows the dependence of the frequency (f , Hz) of oscillations on the concentration of methyl, ethyl, n -propyl, n -butyl, and n -pentyl alcohols. The average frequency was measured in the region where rhythmic oscillations were observed, and experiments were performed at each concentration at least three times. As can be seen, the frequency increased sharply when the concentration of an alcohol exceeded a critical value. The concentration

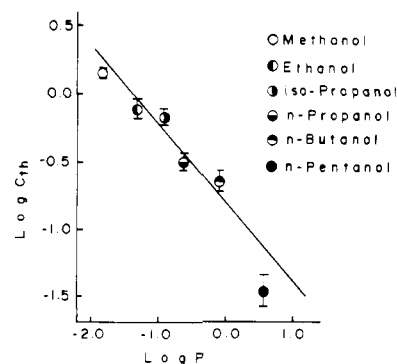


Figure 7. Relationship between $\log C_{th}$ and $\log P$. C_{th} is the concentration at which the frequency of oscillations changes most sharply in Figures 4 and 5. P is the partition coefficient between benzene and water. Vertical bars represent 95% confidence intervals of means.

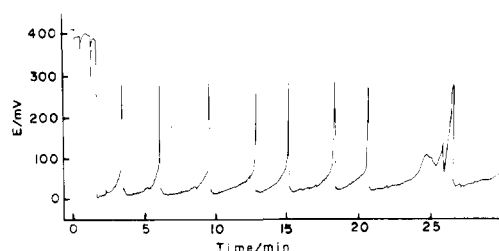


Figure 8. Oscillation of electrical potential with 0.1 M benzyl alcohol in the left aqueous phase. Other experimental conditions were the same as for Figure 2.

dependence of the oscillations differed for n -propyl alcohol and isopropyl alcohol, as is also shown in Figure 5: the critical concentration for induction of oscillations was lower for n -propyl alcohol than for isopropyl alcohol.

Figure 6 shows that the concentration-dependence of the logarithmic response (R) apparently follows a similar relationship to that of Langmuir's adsorption isotherm when the amount of adsorbed substance is replaced by R (the logarithm of the frequency of oscillation; $R = \log f$)

$$C/R = C/R_{th} + 1/(KR_{th}) \quad (1)$$

where R_{th} is the maximum response corresponding to the maximum amount of adsorption in Langmuir's adsorption isotherm and K is an apparent equilibrium constant. It is interesting that a similar relation has been reported for the concentration dependence of the magnitude of the nerve response in taste reception.¹²

Influence of Hydrophobicity of Alcohols. The logarithm of the threshold concentration C_{th} is plotted against the hydrophobicity of alcohols in Figure 7. The C_{th} is the concentration where the frequency of oscillations changed most sharply in Figures 4 and 5. The hydrophobicities of alcohols are represented as the logarithms of their partition coefficients, P , between benzene and water.¹³ It is noteworthy that $\log P$ is proportional to the free energy of transfer of the alcohol from water to a hydrophobic environment. As shown in Figure 7, the following relation apparently holds for the aliphatic alcohols, including isopropyl alcohol, used in this experiment,

$$\log C_{th} = -a \log P + b \quad (2)$$

where a and b are constants. It should be stressed that a similar relationship has been found for taste reception.^{14,15}

Effect of Aromatic Alcohols. Figure 8 shows the oscillations of electrical potential observed when 0.1 M benzyl alcohol was used instead of ethanol under otherwise the same experimental conditions as for Figure 1. It is interesting that the mode of oscillation is quite different from that with aliphatic alcohols

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(Figures 1 and 2): the spikes were generated toward a higher potential with benzyl alcohol but toward 0 mV with aliphatic alcohols.

Discussion

Recently we found⁹ that sustained, regular oscillations of electrical potential occurred in a two-phase system of a solution of 1.25 mM picric acid in 2-nitropropane and an aqueous solution of 5 mM CTAB. When the solutions were not stirred, the oscillations showed a constant period (0.5–2 min) and continued for 1–2 h, whereas no oscillations occurred when the solutions were stirred. It is interesting that a similar phenomenon was observed in a two-phase system⁹ consisting of organic and aqueous solutions and also in a liquid-membrane system¹⁰ consisting of aqueous, organic and aqueous solutions as we reported previously and as shown in the present work. It is noteworthy that in these experiments the concentrations of solutes were in a kind of non-equilibrium state; i.e., the hydrophilic substance picric acid was dissolved in the organic phase and the hydrophobic substance, CTAB, was dissolved in the aqueous phase. In this sense, the phenomena observed in the two-phase system and in the liquid membrane can be regarded as periodic structure formation at or near interfaces in a non-equilibrium state.

Mechanism of the Electrical Oscillation. The electrical potential, $\Delta\psi$, between the two aqueous phases in a liquid membrane is considered as the algebraic sum of two phase-boundary potentials on the right and left interfaces (ΔE_r , ΔE_l) and a different potential within the organic phase (ΔE_d).

$$\Delta\psi = \Delta E_r + \Delta E_d + \Delta E_l \quad (3)$$

ΔE_r , ΔE_d , and ΔE_l cannot be measured separately, but it seems meaningful to discuss the contributions of these components to the total membrane potential. As shown in Figures 2–4, the potential, $\Delta\psi$, was 300–400 mV in the resting state and decreased abruptly toward 0 mV when a pulse was generated. Probably this large change in potential is due mainly to change in the phase-boundary potential (ΔE_r and/or ΔE_l), the contribution of change in the diffusion potential (ΔE_d) being relatively small. This concept is based on the idea that if the change in diffusion potential were important for the oscillation, ionic conductance for a specific ion should change abruptly. With a thin membrane, such as a lipid bilayer membrane, it is widely believed that an abrupt change can be generated by an on-and-off effect of a "gate" or "channel" molecule present within the membrane. However, in a thick liquid membrane there is no gate or channel that could change the ionic conductance across the organic phase abruptly, and thus the change of the diffusion potential is probably small.

Next the origin of the phase-boundary potential between the organic and the right aqueous phases should be considered. In the aqueous phase, CTAB is present in solution as CTA^+ and Br^- and CTA cations form micelle. It is known that the critical micelle concentration (cmc) of CTAB is 9.2×10^{-4} M at 25 °C.¹⁶ When the aqueous phase comes in contact with the organic phase, CTA cations, which are mainly present as micelles, move toward the interface and become oriented in a manner such that their hydrophilic ammonium groups are in the aqueous phase and their hydrophobic alkyl chains are in the organic phase. With an increase in concentration at the interface, the CTA cations form a monolayer. This means that the organic phase becomes positive with respect to the aqueous phase.

Mingins et al.¹⁷ studied the potential difference, phase-boundary potential, due to spread monolayers of cetyltrimethylammonium chloride at the interface between an aqueous solution containing NaCl and a polar oil, such as nitrobenzene. They found that the potential difference, phase-boundary potential, was 150–200 mV, and it increased with a decrease in the concentration of NaCl. The positively charged interface where CTA cations are present as a monolayer structure will attract anions such as picrate and

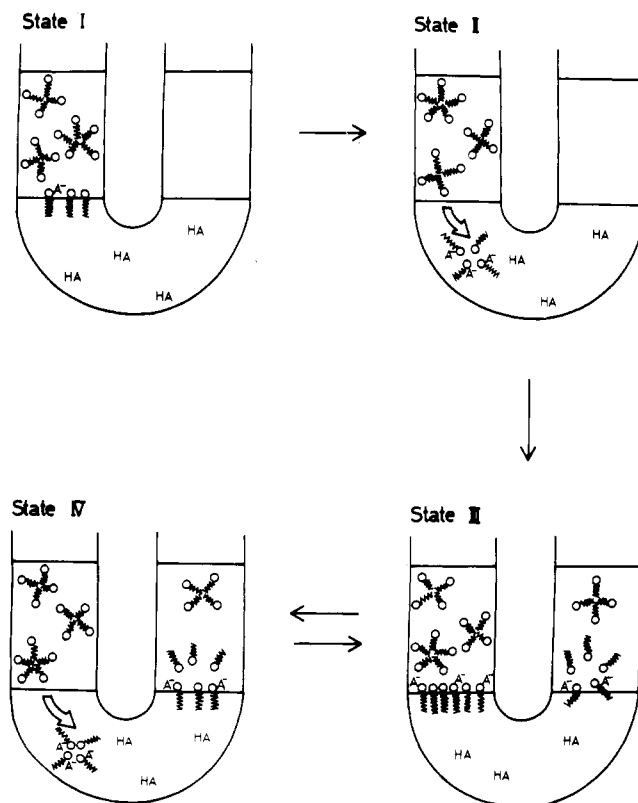


Figure 9. Schematic representation of the mechanism of oscillation: State I, induction period; State II, first impulse; States III = IV, oscillation.

chloride and repulse cations. According to the theory of Gouy–Chapmann,¹⁸ the spatial distribution of the electrical potential and the concentrations of cations and anions follow the Poisson equation and the Boltzmann distribution law, respectively. For a flat membrane surface with a uniform surface charge density, σ , the potential difference between the membrane surface and the bulk solution of a 1:1 type electrolyte, $\Delta\psi$ (phase-boundary potential), is given by

$$\Delta\psi = \frac{2kT}{e} \sinh^{-1} \frac{A\sigma}{\sqrt{C}} \quad (4)$$

where k is the Boltzmann constant, T is the absolute temperature, e is the electric charge, and C is the bulk aqueous concentration of electrolyte. $A = (1/8N\epsilon_0\epsilon_r kT)^{1/2}$, where N is Avogadro's number, ϵ_r is the dielectric constant, and ϵ_0 is the permittivity of free space. When σ is 0.01 Å^{-2} ,¹⁷ C is 5 mM, and ϵ_r is 78.3 (the value of water), $\Delta\psi$ becomes ca. 190 mV at 25 °C.

In our experiment, the compositions of the electrolytes were more complicated. Thus it is difficult to calculate the precise value of the voltage according to the theoretical equation. However, we assume that micelle formation decreased the "effective" concentration of the electrolytes and that this resulted in more than a 190-mV increase of the phase-boundary potential. In this connection, it is noteworthy that Colacicco¹⁹ observed that the electrical potential at a water–pentyl alcohol interface reached 220 mV in the presence of 10 mM CTAB in water and that the potential decreased with an increase of the concentration of KCl or CaCl_2 ions in the water. From these results, he concluded that the observed oil/water potentials were not diffusion potentials but were produced by the adsorption of CTA cations at the interface.

The resting potential of 250–350 mV is thus mainly attributable to the phase-boundary potential induced by the formation of a monolayer of CTA cations at the interface. The abrupt decrease

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of the potential seen in Figures 2–4 could be induced by rapid destruction of the monolayer structure. Previously, we⁹ proposed a mechanism of successive formation and abrupt destruction of the monolayer structure. The oscillation described in this report may be explained in a similar manner, as shown schematically in Figure 9. After the induction period (State I), a first pulse is generated (State II) and repeated interchange of States III and IV occurs.

State I: CTA cations, which are mainly present as micelles in the aqueous phase, move toward the interface and become situated at the interface. Simultaneously molecules of picric acid move toward the interface and dissolve in the aqueous phase. Thus the concentrations of CTA cations and picrate anions around the interface increase gradually, and CTA cations tend to form a monolayer at the interface.

State II: When the concentration of CTA cations at the interface reaches a critical value, CTA cations are abruptly transferred to the organic phase with formation of inverted micelles.

State III: When the concentration of CTA⁺ on the interface decreases to a lower critical value, accumulation of CTA⁺ on the interface begins again with gradual formation of a monolayer.

State IV: When the concentration of CTA⁺ increases to an upper critical value, abrupt transfer of CTA cations to the organic phase occurs.

Effects of Alcohols on the Oscillation. In the Experimental Section it was shown that the dependence of the frequency of oscillations on the concentration of an alcohol apparently follows the relationship of Langmuir's adsorption isotherm (eq 1). It was also found that the critical concentration needed to induce the oscillation depends on the hydrophobicity of the alcohol (eq 2). These effects of alcohols on the oscillation require consideration. It is well known that alcohols decrease the cmc of amphiphilic molecules.²⁰ This decrease is due to incorporation of the alcohol into the micelle, which changes the manner of aggregation of the amphiphilic molecules. Alcohols certainly affect the structure of the CTA⁺ monolayer on the interface.²¹ They may also influence the rate of migration of CTA cations from the aqueous phase to the interface and from the interface to the organic phase. These effects of alcohols may induce change in the frequency of oscillations.

The degree of incorporation of alcohols into aggregates of CTA⁺ (i.e., the monolayer on the interface, micelles in the aqueous phase, and inverted micelles in the organic phase) probably increases with an increase in the hydrophobicity of the alcohols. If so, this could be related to the fact that the hydrophobicity of alcohols is closely related to the frequency of oscillations.

Comparison with Biological Chemoreceptive Membranes. Application of chemical stimuli to a receptor organ induces nerve impulses. The mechanism of generation of this response is a very interesting problem. In 1954 Beidler¹² proposed a hypothesis to

explain taste stimulation based on his finding that the relationship between the magnitude of integrated electrical response of the taste and the concentration of a stimulant satisfies Langmuir's adsorption isotherm; he proposed that adsorption of a chemical stimulant to a taste receptor induces stimulation of the associated sensory neuron. Many lines of evidence support this adsorption hypothesis of taste reception, but the details of the mechanism are still unknown. In the liquid membrane the logarithm of the frequency apparently followed a relationship similar to that of Langmuir's adsorption isotherm, suggesting that the response of the liquid membrane resembles that of the biological chemoreceptive membrane, though details of each mechanism should be different.

Though this study is only a beginning, it indicates the possibilities of developing a new type of chemical sensor capable of distinguishing various chemical substances on the basis of information on the frequency and the shape of impulses.

Conclusions

(1) Sustained oscillations of electrical potential were generated in a liquid membrane consisting of an oil layer, nitrobenzene containing picric acid, imposed between two aqueous phases, one of which contained CTAB and an alcohol.

(2) The frequency of oscillations increased with an increase in the concentration of the alcohol. The logarithm of the frequency apparently obeyed a relationship similar to that of Langmuir's adsorption isotherm.

(3) The threshold concentration needed to induce the oscillation decreased with an increase in the hydrophobicity of the alcohol. The relationship between the critical concentration and the hydrophobicity can be represented by the following equation

$$\log C_{th} = -a \log P + b$$

where C_{th} is the concentration where the oscillations change sharply, P is the partition coefficient of the alcohol between benzene and water, and a and b are constants.

(4) The oscillatory phenomenon can be explained by a mechanism of repetitive formation and destruction of a monolayer of CTA⁺ at the interface.

(5) The manner of the electrical response of the liquid membrane resembles that of biological chemoreceptive membranes.

(6) The possibility of developing a new type of chemical sensor that can distinguish various chemical substances on the basis of information on the frequency and shape of impulses is suggested.

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Registry No. CTAB, 57-09-0; methanol, 67-56-1; ethanol, 64-17-5; isopropyl alcohol, 67-63-0; *n*-propyl alcohol, 71-23-8; *n*-butyl alcohol, 71-36-3; *n*-pentyl alcohol, 71-41-0; benzyl alcohol, 100-51-6; nitrobenzene, 98-95-3; picric acid, 88-89-1.

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