Biophysical Chemistry, 34 (1989) 201–210
Elsevier

BIOCHE 01381

Characteristic response to taste stimuli of the intensities of higher harmonics in an electrochemical oscillatory system

Satoshi Nakata [a], Kenichi Yoshikawa [b], Masaru Shoji [b], Hiroshi Kawakami [c] and Toshio Ishii [d]

[a] *Department of Chemistry, Nara University of Education, Takabatake-cho, Nara 630,* [b] *College of General Education, Nagoya University, Nagoya 464-01,* [c] *Department of Electronic Engineering, Faculty of Engineering, University of Tokushima, Tokushima 770 and* [d] *School of Dental Medicine, Tsurumi University, Tsurumi, Yokohama 230, Japan*

Received 11 April 1989
Revised manuscript received 29 May 1989
Accepted 5 June 1989

Nonlinearity; Electrical oscillator; Sensor, chemical

In general, the electrochemical characteristics of solid/liquid or liquid/liquid interfaces are highly nonlinear, i.e., the capacitance changes markedly according to the applied voltage. In this paper, we propose a novel method for evaluating these nonlinear characteristics quantitatively. That is, a sinusoidal voltage source is applied to a test solution and the waveform of the output current is analyzed by Fourier transformation. It is shown theoretically that higher harmonic components in the Fourier transformation afford us useful information on nonlinear behavior. It is stressed that our technique is entirely different from the classical impedance method, i.e., nonlinear components of the impedance can be evaluated in our method, having been ignored previously in the classical impedance measurement. As an application of this method, we have studied the effect of taste compounds on the intensities of the higher harmonics, using an electrochemical cell containing an aqueous solution of sodium oleate. It has been found that the intensities of the higher harmonics exhibit characteristic changes upon the addition of taste compounds, the change being dependent upon the taste category. The characteristic response to taste compounds in the electrochemical nonlinearity is discussed in relation to the experimental trend of the dynamic isotherm for oleic acid at an air/water interface.

1. Introduction

Living organisms can distinguish between and quantify many chemical species. In taste and olfaction, electrical impulses in the nerve are generated at sensory organs by chemical stimuli; i.e., chemical information on the chemical structure and concentration is converted into frequency and frequency modulation of nerve impulses [1–6]. On the other hand, chemical sensors developed so far, e.g., pH electrodes, measure the activities of test ions in solutions as d.c. voltage differences generated by the electrodes. Excellent studies have been carried out on ion-selective electrodes, and useful electrodes have been devised. However, if one wishes to detect thousands of organic and/or biological molecules, one must develop thousands of selective electrodes [7]. Regrettably, this is an almost impossible task. Therefore, most researchers would agree that information other than the d.c. value generated on the electrode should be utilized. Along these lines, measurement of the 'complex impedance', in other words, the determination of the conductance and capacitance, is expected to be of value. In applying the measurement of impedance to chemical analysis, there exists a severe problem, i.e., the observed value of

Correspondence address: K. Yoshikawa, College of General Education, Nagoya University, Nagoya 464-01, Japan.

0301-4622/89/$03.50

the impedance is not satisfactory with respect to its reproducibility. The poor reproducibility is attributable to a change in the distance between the electrodes, the presence of bubbles on the electrode, hysteresis due to the adsorption of various chemicals onto the electrode, and the nonlinear characteristics of electrochemical systems.

We have currently been studying artificial excitable membranes [8–11]. Using these artificial membranes, it has been demonstrated that various chemical species can be distinguished and quantified based on the information yielded by the amplitude, frequency, shape and modulation of electrical oscillations. Such an interesting oscillatory phenomenon is connected with the nonlinear electrochemical behavior of artificial membranes. Under these circumstances, we have observed that the use of information resulting from electrochemical nonlinearity is promising for the development of chemical sensors. Recently, we have reported a new sensing system that is excited by connection to an external nonlinear electric circuit [12–14]. Using this sensing system, one can analyze inorganic ions [12] and polysaccharides [13], and monitor bacterial growth [14]. However, in this system the precise physical meaning of the modulation of electrical oscillations remains unclear, although the modulation of oscillations is expected to be related to the nonlinearity of the test electrochemical systems.

As an extension of these studies, in the present article we report a novel sensing method. The principle of this method is to characterize the electrochemical nonlinearity by analysis of the higher harmonics obtained from the Fourier transform of the output current in response to an applied sinusoidal voltage. Our method is similar to a harmonic analysis employed in an electrophysiological study of squid nerve fibers [15]. Here, it should be noted that the higher harmonics arise solely from the nonlinearity of the membrane [16]. We show that the reproducibility of this method is excellent and that its application shows promise for the development of chemical sensors. In this report, we describe the principle of this sensing method, together with the results obtained from practical application in the detection and quantitation of various taste compounds.

2. Experimental

Analytical grade sodium oleate was obtained from Sigma. NaCl, HCl, sucrose, nicotine and other chemicals were purchased from Nakarai (Kyoto). The water used in this study was distilled and then purified with a Millipore Milli-Q filtering system, which was maintained at a resistivity of 18 MΩ cm.

Electrical measurements were performed in the apparatus shown schematically in fig. 1. Genuine sinusoidal wave (frequency, 1 Hz; peak-to-peak voltage, 1 V) was generated using a model 459 AL waveform generator (Kikusui Electronics, Japan). Sine wave of precise voltage was applied to the electrochemical system using a potentiostat (model HA-10 R1G, Hokuto Denko, Japan). A two-electrode cell was employed and the ohmic drop arising from solution resistance in the cell was compensated by positive feedback from the potentiostat. The a.c. voltage waveform applied to the test solution and the output current waveform were successively stored in an NEC PC-9801 personal computer (NEC, Japan), and then Fourier-transformed to the frequency domain. Platinum wire (length, 25 mm; diameter, 0.5 mm) was used as the working electrode, with an Ag/AgCl electrode as the reference electrode. Dynamic surface tensions [17] were measured with a trough equipped with a couple of moving teflon blades and an electronic balance (CAHN/Ventron, Cerritos, CA) (fig. 8). Surface areas were changed successively, between 10 and 50 cm^2, by moving the teflon blades with a cycle of 72 s. When the surface pressure (π)-surface area (A) curve began to trace

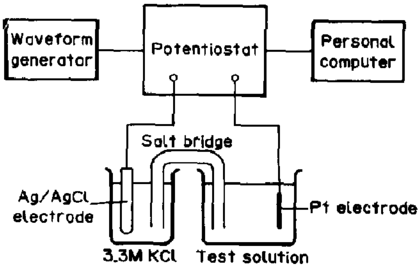


Fig. 1. Diagram of the experimental apparatus used for measuring electrochemical nonlinearity.

a single closed line after several cycles, the curve of the dynamic surface tension was recorded. All measurements were performed at 20 ± 1° C.

3. Theory

Capacitance is generally dependent upon the applied voltage in electrochemical systems. We shall now discuss this nonlinear property of the capacitance. According to the Gouy-Chapman theory of a diffuse double layer of ions formed around the electrode surface, differential capacitance, C_d is given as follows [18].

$$C_d = \left(\frac{2z^2e^2\epsilon\epsilon_0 n^0}{kT}\right)^{1/2}\cosh\left(\frac{ze\phi_0}{2kT}\right) \qquad (1)$$

where z denotes the (signed) number of units of electronic charge, e the elementary charge, ϵ the dielectric constant of the medium, ϵ_0 the permittivity of free space, n^0 the number concentration of each ion in the bulk phase, k Boltzmann's constant, T the absolute temperature (K), and ϕ_0 the electrostatic potential (V).

Eq. 1 indicates that the capacitance due to the electrical double layer of the ions is apparently dependent upon the voltage. When an external sinusoidal voltage is applied to the capacitor with the characteristics given by eq. 1, the output current should deviate from a sinusoidal waveform. Fig. 2 shows a numerical simulation of the distortion of the output current. Here, it is important to note that the degree of distortion can be evaluated from the relative intensities of the higher harmonics in the Fourier transformation. From these considerations, it becomes apparent that the output current in an electrochemical system generally contains higher harmonics, as shown in scheme 1.

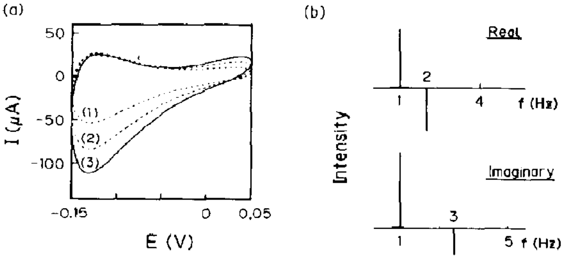
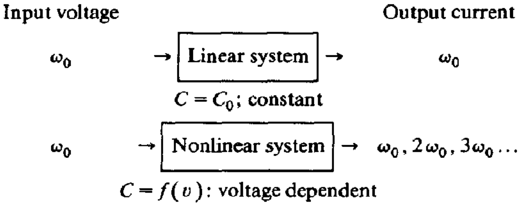


Fig. 2. Simulation of the characteristics of an electrical double layer on the electrode surface in a 1 : 1 electrolyte solution: (a) current-voltage curves for (1) 5 mM electrolyte of resistance 9 kΩ, (2) 10 mM and 4.5 kΩ, and (3) 15 mM and 3 kΩ; and (b) Fourier transformation of curve 3 in panel a with a period of 1 s per cycle.



Scheme 1. Higher harmonics ($2\omega_0$, $3\omega_0$, ...) are generated in an electrochemical system with nonlinear capacitance.

Although eq. 1 is not sufficient for interpreting the details of the electrical properties in practical electrochemical systems, it is generally accepted that the output current contains higher harmonics. From the higher harmonics of the output current, one can quantify the nonlinear characteristics of the electrochemical system.

Here, it may be important to stress the significance of measuring the nonlinear characteristics of capacitance. Many thorough investigations have been carried out on the capacitive component of the diffuse double layer. It has been shown that the observed capacitance in an electrochemical system is dependent on the frequency of the applied voltage. Such behavior could be interpreted by taking into consideration the equivalent circuit with capacitors and resistors in series and/or in parallel. With these equivalent circuits, one can interpret the dependence of the capacitance on the frequency of the applied voltage. Here, it should be noted that no higher harmonics will appear, even with an electrical circuit composed of many capacitors and resistors, if these capacitors and resistors have only 'linear characteristics'. Nonlinear characteristics only produce the higher harmonics with the application of a sinusoidal voltage of a single frequency.

The measurement of higher harmonics affords us abundant data concerning an electrochemical system. The classical impedance measurement has been carried out based on the assumption of a 'linear system'. Since the relative intensities of the Fourier components, the fundamental and higher harmonics vary proportionally to the area of the electrode, the normalized value of the intensities of the higher harmonics ($n\omega_0$) with respect to the fundamental component (ω_0) becomes independent of the electrode area. The change in electrode surface area, arising from a difference in the manner of immersion of the electrode during handling or from the presence of air bubbles formed on the electrode, is thus compensated through this normalization procedure, which results in greater reproducibility. In addition to this improvement, the increase in information regarding the electrochemical system is also quite useful for its analysis.

In order to clarify the relationship between the intensities of the higher harmonics and the electrochemical nonlinearity, let us now discuss the capacitance which possesses simple nonlinear characteristics, as expressed by eq. 2:

$$Q = C_0 V + \tfrac{1}{2} C_1 V^2 + \tfrac{1}{3} C_2 V^3 \qquad (2)$$

When a sinusoidal voltage is applied to a system of parallel circuits, with this nonlinear capacitor and a resistor (fig. 3), the output current is described by eq. 3 with the assumption that C is independent of time (see the appendix):

$$\begin{aligned} I(t) = {} & \frac{E_0}{R} + \left(C_2 E_0^2 + C_1 E_0 + C_0 + \tfrac{1}{4} C_2 E_1^2\right) \\ & \times \omega_0 E_1 \cos \omega_0 t \\ & - \tfrac{1}{4} \omega_0 C_2 E_1^3 \cos 3\omega_0 t + \frac{E_1}{R} \sin \omega_0 t \\ & + \tfrac{1}{2}(2C_2 E_0 + C_1) \omega_0 E_1^2 \sin 2\omega_0 t \qquad (3) \end{aligned}$$

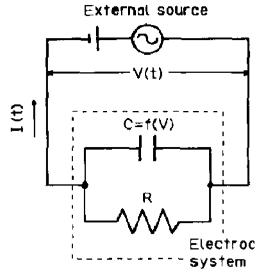


Fig. 3. Model circuit used for the theoretical simulation.

When the function $I(t)$ is Fourier transformed, the coefficients of the sine and cosine functions correspond to the real and imaginary components, respectively (see fig. 2b). From this relationship, it is apparent that the second and third harmonics correspond to the first and second derivatives, respectively, of the nonlinearity of the capacitance. In addition, hysteresis [19,20] may contribute to the nonlinear property of an electrochemical system. It should thus be noted that hysteresis also contributes to the intensities of the higher harmonics. The effect of hysteresis on the relative intensities of higher harmonics may be evaluated by taking into account the rate of diffusion of electrolytes near the interface. We are currently attempting further development of a theory including hysteresis. In the present study, we applied an external a.c. voltage of rather low frequency (1 Hz). The reason for this choice is that the dielectric property of the bulk solution contributes to the capacitance with higher frequency, and thus the capacitive component of the diffuse double layer near the electrode surface could be observed only with low frequencies [21,22].

3. Results and discussion

3.1. Characteristic current-voltage curve for taste compounds

Recently, we have succeeded in distinguishing between substances belonging to the four basic taste categories by observing the difference in patterns of self-oscillation in a water-oil-water liquid membrane containing sodium oleate [11]. In the present paper, we describe the change in the electrochemical characteristics of a solution of sodium oleate induced by the addition of various taste compounds, representing the four basic taste categories, sweet, sour, salty and bitter, having paid particular attention to quantitative detection of any nonlinearity in the system.

Fig. 4 exemplifies the change of the I–V (I, current; V, sinusoidal voltage of 1 Hz) curve in a 1 mM aqueous sodium oleate solution caused by the addition of the following taste compounds, (a)

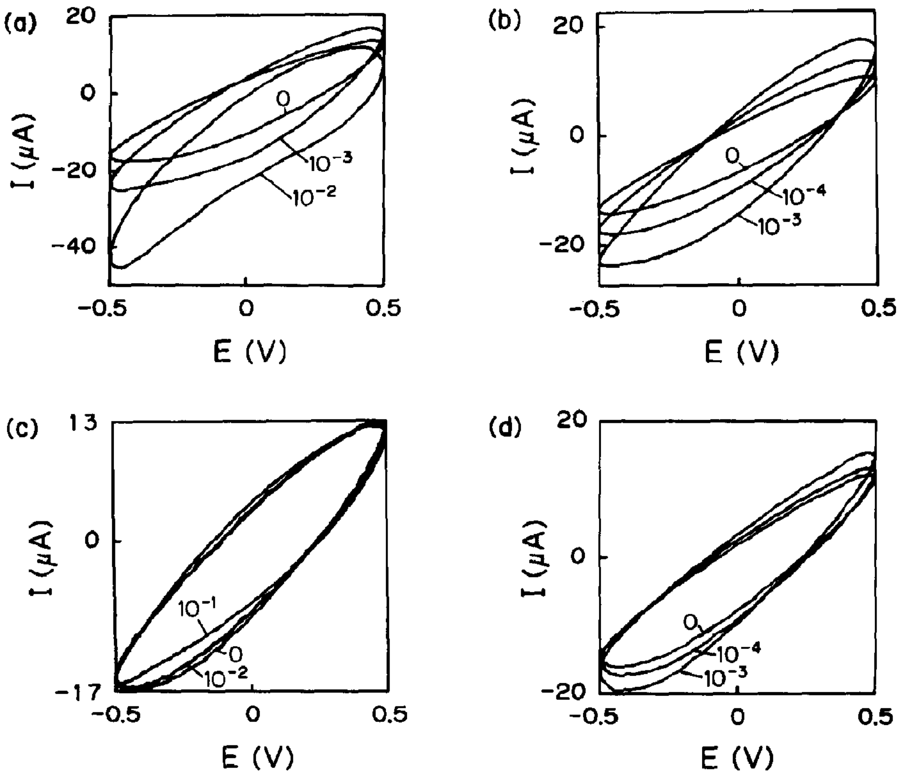


Fig. 4. Alterations in current-voltage curve with addition of taste compounds to 1 mM aqueous sodium oleate solution. Frequency of sinusoidal voltage, 1 Hz. (a) Hydrochloric acid, (b) sodium chloride, (c) sucrose, (d) nicotine. Numbers beside curves denote concentrations (M) of taste compounds.

hydrochloric acid (sour), (b) sodium chloride (salty), (c) sucrose (sweet), and (d) nicotine (bitter). It is interesting to note that the effects of each class of taste compounds are apparently different. In order to analyze the characteristics of the I–V curve quantitatively, the trace of the output current vs. time was Fourier transformed to the frequency domain (fig. 5). Here, R_n and I_n correspond to the intensity of the real part (conductance) and imaginary component (capacitance) of the n-th harmonics, respectively. It was found that the relative intensities vary characteristically according to the concentration of taste compounds. In order to minimize the experimental error, the intensities of the second and third harmonics are given as normalized values relative to the imaginary component of the first harmonics, I_1, which corresponds to the linear component of the capacitance. The linear components showed relatively large experimental errors (approx. 30–50%) on each experimental run. With the normalization procedure, the errors in the relative intensities were found to be less than 10% for all measurements in this study (fig. 5).

Fig. 6 shows a schematic representation of the characteristic response of the intensities of the higher harmonics to the various taste compounds which have been categorized into four different taste sensations. It has been confirmed that the form response clearly depends on the taste category of the compound. We have examined at least three compounds for each category: e.g., acetic, tartaric and citric acids (sour); LiCl, KCl and NH_4Cl (salty); and theobromine, quinine hydrochloride and caffeine (bitter). Among these compounds, only the effect of nicotine differed slightly

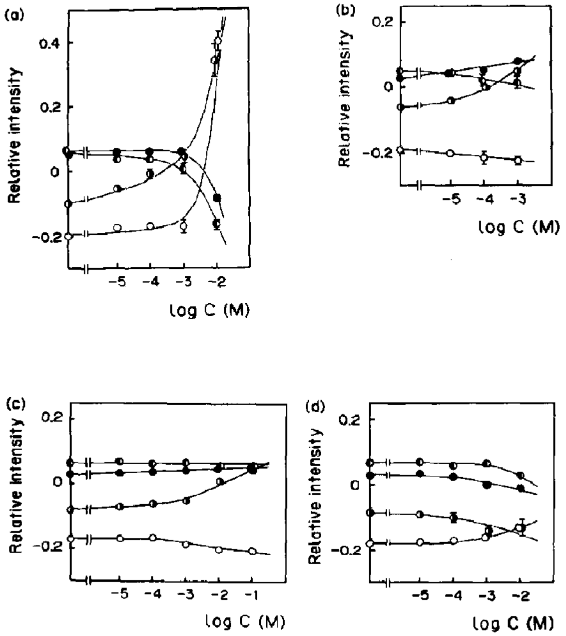


Fig. 5. Variation in relative intensity (R_n/I_1 or I_n/I_1) in the Fourier transformation for the measurement shown in fig. 4. (a) Hydrochloric acid, (b) sodium chloride, (c) sucrose, (d) nicotine. (◑) R_2/I_1, (◐) R_3/I_1, (○) I_2/I_1, (●) I_3/I_1. R_n, intensity of the real part (conductance) of the n-th harmonics; I_n, intensity of the imaginary part (capacitance) of the n-th harmonics.

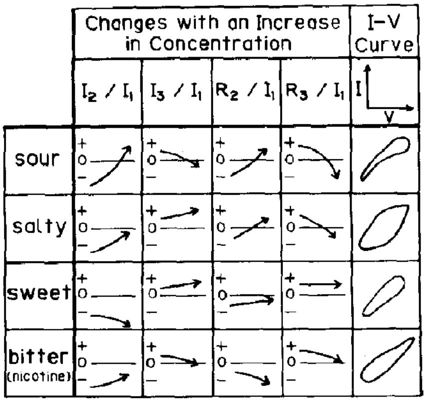


Fig. 6. Schematic representation of the characteristic responses to the magnitude of the higher harmonics.

from those of the other bitter compounds, i.e., the increase in area of the closed curve was not as large. From the data obtained in measurements of these compounds, it becomes apparent that the sensing method reported herein is quite useful for distinguishing and quantifying various chemical substances when measurements based on higher harmonics are employed.

3.2. Nonlinearity of capacitance in relation to the higher harmonics

Fig. 7 shows a simulated capacitance-voltage curve based on the experimental results on Fourier transformation of the current-voltage curve. In this computer simulation, the components of the second and third harmonics have been evaluated using eq. 3 and those of the higher harmonics, such as the fourth and fifth harmonics, have been ignored. It is clearly demonstrated in fig. 7 that the nonlinearity of capacitance changes in a characteristic manner on addition of taste compounds. Here, it should be noted that, in the classical impedance measurements, the value of the capacitance observed exhibits time-dependent variations and generally shows marked hysteresis.

We shall now discuss the characteristic alterations in nonlinearity of the capacitance. Sour compounds, e.g., hydrochloric and tartaric acids, lower the pH and affect the degree of dissociation of the carboxyl group on oleic acid. When this dissociation is reduced, the adsorption of negative charge density onto the electrode, caused by anionic oleate onto the electrode surface, should decrease and the manner of aggregation of oleate anion and/or neutral oleic acid should change. The variation in electron charge density on the electrode surface may alter the shape of the voltage-dependent capacitance, as shown in fig. 7a.

Salty compounds, e.g., NaCl and NH_4Cl, affect the electronic diffuse double layer around the surface of the electrode. According to Gouy-Chapman theory [18], which draws on the assumption of a Boltzmann distribution and Maxwell-Planck relationship, the capacitance should increase with rising concentration of electrolyte. Fig. 7b corresponds to this expectation. However, from Gouy-Chapman theory it is expected that the second derivative of the curve for voltage-dependent capacitance should be positive. In contrast, the curve in fig. 7b shows that the second derivative

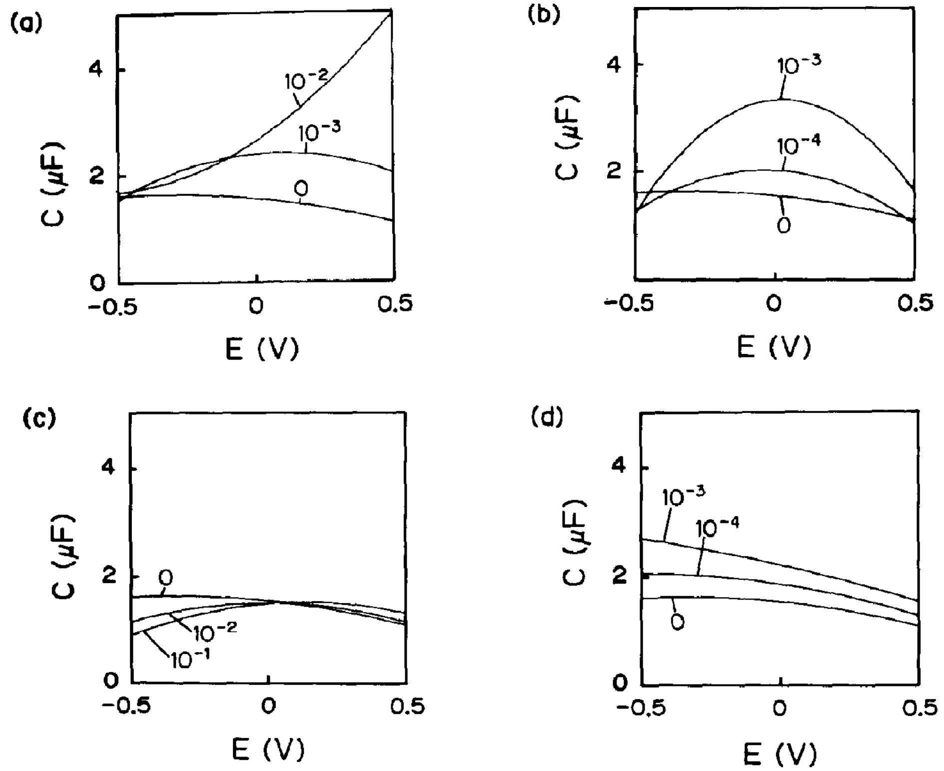


Fig. 7. Capacitance-voltage curve deduced from the Fourier-transformed spectra. (a) Hydrochloric acid, (b) sodium chloride, (c) sucrose, (d) nicotine. Concentrations (M) of taste compounds are denoted by the numbers beside the curves.

becomes more negative on addition of salty compounds. This discrepancy between theory and experiment may be due to aggregation or adsorption of oleate anion, since the derivation of Gouy-Chapman theory is based on the assumption that there are only fully ionized, nonaggregated ions, which do not adhere to the electrode surface.

The effect of sweet compounds on the nonlinear characteristics of the capacitance is not as marked, however, alterations apparently do occur. It is generally believed that sweet compounds change the structure of the water cluster [23,24]. Such changes in the water cluster may affect the manner of adsorption and/or aggregation of oleate molecules.

It is assumed that bitter compounds, such as nicotine, are incorporated mainly into the hydrophobic region of aggregated lipids or surfactant molecules [6]. It is therefore expected that bitter compounds induce greater adsorption of oleate ion onto the electrode surface, possibly with the capacitance subsequently increasing, as seen in fig. 7d.

At present, the above interpretation of the nonlinear characteristics as being dependent on the taste category of the test compounds is rather primitive. However, it does at least appear that compounds belonging to different taste categories affect the nonlinear property of the electrode in different ways. With adequate modification of the electrode surface it should be possible to improve the selective response of the nonlinearity to various chemicals. Studies along this line are being planned.

3.3. Effects of taste compounds on the dynamic π-A characteristics

As shown in section 3.2, each taste compound alters the nonlinearity of the electrode in a manner that is characteristic of the taste category. We have found [11] that a rhythmic, sustained electrical oscillation is generated for a water-oil-water liquid membrane which contains sodium oleate in one of the aqueous phases. The frequency, amplitude and shape of the electrical oscillations were found to vary characteristically after addition of various taste compounds to the aqueous phase containing sodium oleate. The dependence of electrical nonlinearity on the nature of the taste compounds may, therefore, be expected to be related to this characteristic change in the liquid membrane. In order to shed light on this relationship and to determine the effect of the mechanism of reaction of taste compounds upon the manner of adsorption and/or aggregation of oleate molecules, we have studied the dynamics of the surface pressure of oleic acid at an air-water interface. Since studies on the dynamic characteristics of interfacial pressure, at both an oil-water interface and an electrode surface are technically difficult, we have investigated the nature of oleate molecules at an air-water interface. The experimental apparatus is schematically shown in fig. 8. In this experimental system, the teflon blade (C) compresses the surface in a periodic manner, i.e., sinusoidally, and the change induced in surface pressure is continuously monitored by using a clean platinum plate (B) attached to an electronic balance, similarly to the method of Bienkouski and Skolnick [17]. Fig. 9 shows typical patterns for the response of the dynamic surface pressure to the addition of taste compounds. The results again demonstrate that taste compounds induce marked alterations in the manner of aggregation at a surface, such changes being dependent upon the taste categories. The molecular mechanism, for the change caused by taste compounds in the manner of aggregation of oleate molecules at an air-water interface, is expected to be similar to that described in section 3.2 for the process of aggregation at the electrode surface.

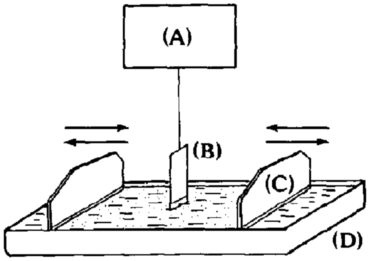


Fig. 8. Diagram of the experimental apparatus used for measuring the dynamic π-A characteristics. (A) Electronic balance, (B) platinum plate, (C) teflon blade, (D) trough.

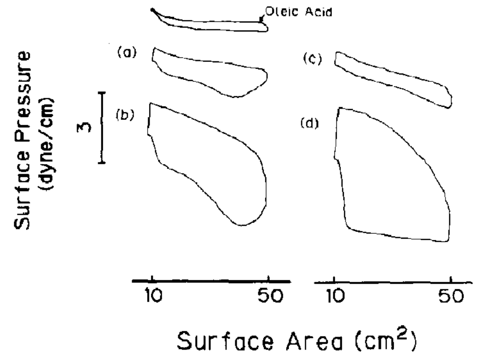


Fig. 9. Characteristic change in dynamic π-A curve caused by addition of taste compounds to aqueous solution containing oleic acid. (a) 10 mM hydrochloric acid, (b) 30 mM sodium chloride, (c) 100 mM sucrose, (d) 1 mM nicotine.

3.4. Relationship between the characteristic response of an artificial system and the mechanism of chemical sensing in living organisms

The rapid advancements recently made in biochemical studies have helped in demonstrating the existence of many receptor proteins. It is quite apparent that living organisms can distinguish specific compounds using these receptor proteins, e.g., pheromones in insects, antigen-antibody interactions, etc. On the other hand, thousands of chemical compounds are present in the surroundings of a living organism. The question then arises as to whether it is possible for living organisms to distinguish between thousands of individual chemicals using thousands of selective receptor proteins. At least, with the chemical sensing of taste and olfaction, it is well known that the selectivity for a chemical substance shown by a single sensing cell is not highly specific but shows a rather broad spectrum [1,2]. On considering the beginning of life on Earth, then if it were a necessary condition that the initial cells possessed selective receptor proteins, living organisms would never have appeared. It is therefore more likely that the earliest forms of life were equipped with a simple chemical-sensing mechanism rather than with many selective receptor proteins. After several thousand million years of evolution, living organisms are now equipped with highly complicated receptor proteins. The present results, together with those reported previously [8–14] from our laboratory, indicate that the aggregation of oleate molecules changes in a manner which is characteristic of the nature of the added taste compounds. Recently, we found [25] that bilayer phospholipid membranes, having an unsaturated alkyl moiety such as an oleyl chain, carry out the function of ion gating even in the absence of a 'channel protein'. This fact is also indicative of the important role played by unsaturated lipids in living organisms with respect to the long evolutionary process. At the present stage, the hypothesis that the main mechanism of taste and olfaction is due to receptor proteins is widely believed. Since the idea presented in this article is still rather primitive, further studies are needed for its justification. We are currently planning to extend our study to phospholipid membranes as a model for biomembranes of receptor cells.

Appendix

Suppose that an electrochemical system is modeled by a nonlinear capacitor C in parallel with a linear resistor R (fig. 3). Let a sinusoidal voltage source $V(t)$ be applied to the circuit as:

$$V(t) = E_0 + E_1 \sin \omega_0 t \tag{A1}$$

Then we obtain

$$I_R(t) = \frac{E_0}{R} + \frac{E_1}{R} \sin \omega_0 t \tag{A2}$$

$$I_C(t) = \frac{\mathrm{d}Q}{\mathrm{d}t} = C\frac{\mathrm{d}V}{\mathrm{d}t}$$

where $C = \mathrm{d}Q/\mathrm{d}V$

$$\begin{aligned} I_C(t) &= C(V)\frac{\mathrm{d}}{\mathrm{d}t}(E_0 + E_1 \sin \omega_0 t) \\ &= (C_2V^2 + C_1V + C_0)\omega_0 E_1 \cos \omega_0 t \\ &= \{C_2(E_0 + E_1 \sin \omega_0 t)^2 \\ &\quad + C_1(E_0 + E_1 \sin \omega_0 t) \\ &\quad + C_0\}\omega_0 E_1 \cos \omega_0 t \\ &= (C_2E_0^2 + C_1E_0 + C_0)\omega_0 E_1 \cos \omega_0 t \\ &\quad + \tfrac{1}{2}(2C_2E_0 + C_1)\omega_0 E_1^2 \sin 2\omega_0 t \\ &\quad + \tfrac{1}{4}\omega_0 C_2 E_1^3(\cos \omega_0 t - \cos 3\omega_0 t) \end{aligned} \tag{A3}$$

where I_R and I_C denote the currents through the resistor and capacitor, respectively. The total current $I(t)$ through the voltage source becomes

$$I(t) = I_R(t) + I_C(t) \tag{A4}$$

Substituting eqs. 2 and A1–A3 into eq. A4, we obtain eq. 3 as:

$$\begin{aligned} I(t) &= \frac{E_0}{R} + (C_2E_0^2 + C_1E_0 + C_0 + \tfrac{1}{4}C_2E_1^2) \\ &\quad \times \omega_0 E_1 \cos \omega_0 t \\ &\quad - \tfrac{1}{4}\omega_0 C_2 E_1^3 \cos 3\omega_0 t + \frac{E_1}{R} \sin \omega_0 t \\ &\quad + \tfrac{1}{2}(2C_2E_0 + C_1)\omega_0 E_1^2 \sin 2\omega_0 t \end{aligned} \tag{3}$$

Acknowledgements

The authors are grateful to Mr. O. Shima for technical assistance. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture and Science.

References

1 D. Roper and J. Atema, Ann. N.Y. Acad. Sci. (Olfaction and Taste IX) 510 (1987).

2 T.E. Finger and W.L. Silver, Neurobiology of taste and smell (Wiley, New York, 1987).

3 R.J. Cherry, G.H. Dodd and D. Chapman, Biochim. Biophys. Acta 211 (1970) 409.

4 E.E. Fesenko, V.I. Novoselov, G.Ya. Pervukhin and N.K. Fesenko, Biochim. Biophys. Acta 466 (1977) 347.

5 N. Koyama and K. Kurihara, Nature 236 (1972) 402.

6 N. Koyama and K. Kurihara, Biochim. Biophys. Acta 288 (1972) 22.

7 G.A. Rechnitz, Chem. Eng. News Sept. 5 (1988) 24.

8 K. Yoshikawa and Y. Matsubara, J. Am. Chem. Soc. 105 (1983) 5967.

9 K. Yoshikawa and Y. Matsubara, J. Am. Chem. Soc. 106 (1984) 4423.

10 K. Yoshikawa, S. Nakata, T. Omochi and G. Collacicco, Langmuir 2 (1986) 715.

11 K. Yoshikawa, M. Shoji, S. Nakata, S. Maeda and H. Kawakami, Langmuir 4 (1988) 759.

12 K. Yoshikawa and T. Omochi, Biochem. Biophys. Res. Commun. 137 (1986) 978.

13 K. Yoshikawa, T. Omochi, T. Fujimoto and H. Terada, J. Colloid Interface Sci. 113 (1986) 585.

14 K. Yoshikawa and T. Ishii, Phys. Org. Chem. 31 (1987) 477.

15 L.E. Moore, H.M. Fishman and D.J.M. Poussart, J. Membrane Biol. 54 (1980) 157.

16 R. FitzHugh, Biophys. J. 42 (1983) 11.

17 R. Bienkouski and M. Skolnick, J. Colloid Interface Sci. 39 (1972) 323.

18 A.J. Bard and L.R. Faulkner, Electrochemical Methods (Wiley, New York, 1980) p. 507.

19 R. de Levie, Chem. Rev. 88 (1988) 599.

20 O. Citri and I.R. Epstein, J. Phys. Chem. 91 (1987) 6034.

21 T. Hanai, D.A. Haydon and J. Taylor, Proc. Roy. Soc. A 281 (1964) 377.

22 T. Hanai, D.A. Haydon and J. Taylor, Theor. Biol. 9 (1965) 278.

23 F. Franks, Water; A comprehensive treatise (Plenum, New York, 1975) vol. 4, p. 519.

24 H.D. Brown, Chemistry of the cell interface, (part B) (Academic Press, New York, 1980) ch. 4.

25 K. Yoshikawa, T. Fujimoto, T. Shimooka, H. Terada, N. Kumazawa and T. Ishii, Biophys. Chem. 29 (1988) 293.