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Characteristics of current induced potential oscillations of a triolein impregnated membrane placed between identical salt solutions

Kei Urabe *, Hiroshi Sakaguchi

National Institute of Materials and Chemical Research, 1-1 Higashi, Tsukuba 305, Japan

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Abstract

Periodic oscillations of electric potential were induced by DC electric current between identical salt solutions separated by a membrane filter impregnated with triolein. The oscillation period was controlled by the base electric potential, and temperature dependence of base conductance and conductance amplitude were both close to that of the electric conductivity of the aqueous salt solution. Effects of salt concentration on membrane conductance and on oscillation characteristics were experimentally investigated, and the lifetime of the membrane and of each oscillation were much improved by regulating the concentration of the salt solution. Moreover, characteristics of oscillation curves could be controlled. All the experimental results could be explained by a model where the oscillation was generated by a periodic change of the diameter of a hole through the membrane, which opened in one of the pores filled with triolein.

Keywords: Oscillation; Triolein; Membrane conductance; Aqueous salt solution

1. Introduction

Several types of self-sustained potential oscillations under constant external forces have been observed in membrane systems such as glass filter [1], liquid membrane with surfactant [2–4], lipid bilayer [5] or filter impregnated with lipid [6–16]. Such oscillatory phenomena observed in membrane systems are interesting and important not only in an analogy with biological functions such as nerve excitation or pacemaker cells, but also in possible practical uses such as sensors or transducers. Fundamental research to clarify the oscillation mechanisms and to obtain better reproducible, stable and controllable oscillations are essential.

For a membrane impregnated with triolein, it has been reported that self-sustained oscillations occurred between equimolar KCl and NaCl aqueous solutions without any external stimuli [12–14]. In previous reports, we showed that stability and reproducibility of the periodic oscillations were improved by applying DC electric current, and that the oscillation periods were dependent on the electric current applied [15,16]. In all of these previous experiments, the salt solutions separated by the filter were equimolar NaCl and KCl.

In this paper, the authors show the experimental results under a condition where the salt solutions on

* Corresponding author.

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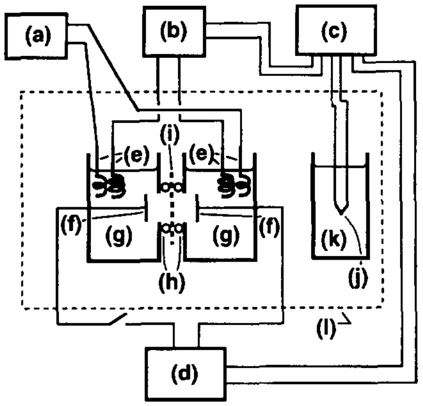

Fig. 1. Experimental set up. (a) Current source (Advantest TR6143), (b) electrometer (Advantest TR8411), (c) pen recorder (Yokogawa LR4210), (d) impedance meter (Delica D51S), (e) Ag/AgCl electrode, (f) Pt-black electrode, (g) aqueous salt solution, (h) silicon rubber gasket, (i) filter impregnated with triolein, (j) thermocouple, (k) aqueous $CaCl_2$ solution for monitoring temperature, and (l) thermostated air bath (Satake SC-T4-70C). The equipment depicted in the dotted cadre were placed in the thermostated air bath.

the two sides of the filter are identical. Subsequently, experimental results on effects of salt concentration and a way to improve stability of the membrane and lifetime of oscillation are shown. A way to control part of the characteristics of oscillation curves is also found out. Relationships between the data obtained and the models of oscillation are discussed.

2. Experimental

The experimental set up is shown schematically in Fig. 1. A cellulose ester filter paper of 8-μm nominal pore size and 150-μm thickness (Nihon Millipore Ltd., type SCWP) was immersed in triolein. Then the filter paper was placed between two acrylic cells filled with identical aqueous salt solutions. Salt and concentration were KCl/0.5 M or NaCl/0.5 M in each experiment as for the results shown in the Section 3.1 and 3.2. Effects of salt concentration on the membrane and oscillation characteristics were investigated for NaCl, whose concentration was varied in a range from 3 mM to 3 M, because oscillations tended to occur more often when a NaCl solution was used. An electric current was applied from a regulated current source through a pair of Ag/AgCl electrodes, and the electric potential between the two solutions was picked up by an electrometer through a pair of Ag/AgCl electrodes and recorded by a pen recorder. Before applying electric current, conductance was monitored by an impedance meter at 120 Hz through a pair of Pt-black electrodes. Experiments were done at a temperature of 19 ± 1°C, except experiments on temperature effects.

3. Experimental results

3.1. General characteristics of oscillations under identical salt conditions

The membrane conductance kept a very low value of 0.5–1 nS (1–2 GΩ in membrane resistance) for several days and no oscillation occurred. After this initial period, the conductance increased stepwise as shown in Fig. 2. At this stage, application of constant electric current induced stable oscillations of the electric potential. An example of oscillation curves is shown in Fig. 3. Oscillation curves of the bistable or sawtooth type were also observed (see Fig. 6 and Ref. [16]).

Generally, once an oscillation began, period and amplitude of each oscillation were stable for at least several hours, and often for several days. The shape of the oscillation curve, range of period, amplitude and/or base membrane conductance differed from one oscillation to another, and usually two or more oscillations were observed sequentially or simultaneously in one sample. Oscillation periods varied from several seconds to an hour. Base membrane conductance (conductance at the higher side of oscillating electric potential) during oscillations, G, and conduc-

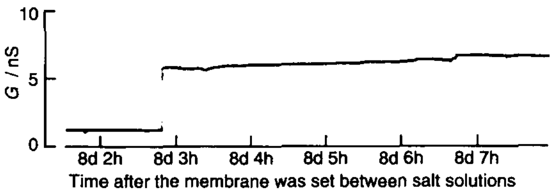

Fig. 2. A stepwise increase of the membrane conductance, which usually occurred several days after the filter had been set between salt solutions of 0.5 M NaCl. Such increase(s) was necessary for appearance of the oscillations.

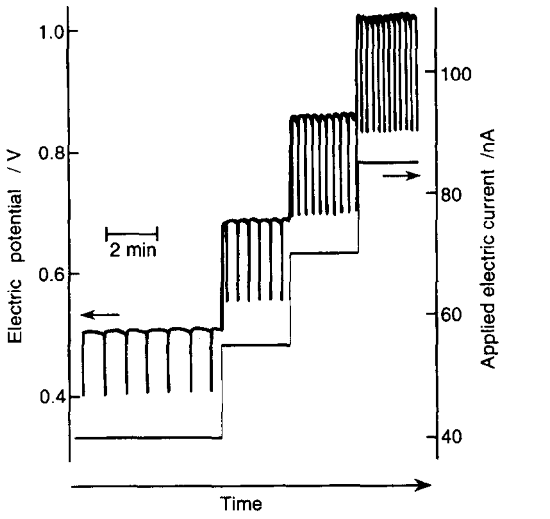

Fig. 3. An example of oscillation curves of downward pulse type shown together with the values of the current applied. Salt solution separated by the filter is 0.5 M KCl.

tance amplitude, ΔG, varied in ranges of 10^{-2}–1 μS and 10^{-3}–10^{-1} μS, respectively, when the solution was 0.5 M NaCl. When the solution was 0.5 M KCl, the ranges of G and ΔG shifted to about 10 times smaller value than the ranges in NaCl solution.

The oscillation periods depended on the current applied (Fig. 3). Plots of the oscillation period versus logarithmic base electric potential followed a straight line for each of oscillations on a logarithmic scale (see Fig. 5). The slope of the line was different from one oscillation to another, usually being between −1.0 and −2.5.

3.2. Temperature dependence

Fig. 4 shows typical Arrhenius plots for G and ΔG. The Arrhenius plots always fitted well to a straight line. Values of the activation energy, calculated from the slopes of the plots, were similar to those of the electric conductivity of the bulk aqueous salt solution, which is 13.8 kJ mol^{-1} for 0.5 M NaCl [17].

Typical period–potential relationships at various temperatures are shown in Fig. 5. The line shifted to the shorter-period side as temperature rose. The shift is larger at low temperatures, and becomes smaller at higher temperatures.

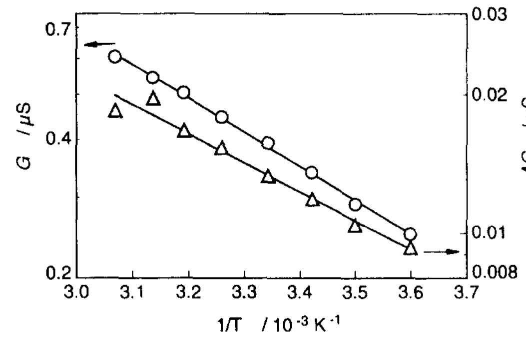

Fig. 4. Arrhenius plots of base conductance, G, and conductance amplitude, ΔG, for the oscillation marked '∗' in Fig. 6. Values of activation energy calculated from the slope are 14.1 kJ mol^{-1} for G and 12.0 kJ mol^{-1} for ΔG, respectively.

3.3. Effect of salt concentration and control of membrane characteristics

3.3.1. Effects of the salt concentration on the stability of the membrane

Oscillations occurred in a wide range of salt concentrations, from 3 mM to 1 M of NaCl. Fig. 6 shows changes of G with time for 3 samples plotted together with oscillation curves observed at each point, when the salt concentration was 0.5 M. The membrane conductance gradually increased, the shape of oscillation curves changed one after the other, and finally the oscillation no longer occurred after 2 or 3 weeks. Generally, G increased more quickly and oscillations were less stable as salt concentration became lower.

On the other hand, when the salt concentration was in the range of 2–3 M, stepwise increases of G

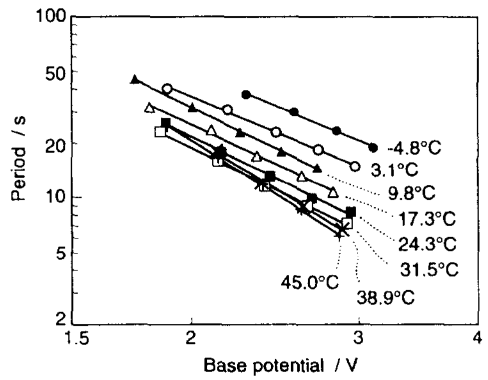

Fig. 5. Plots of oscillation period versus base electric potential, for the oscillation for the oscillation marked '∗' in Fig. 6, under various temperature conditions.

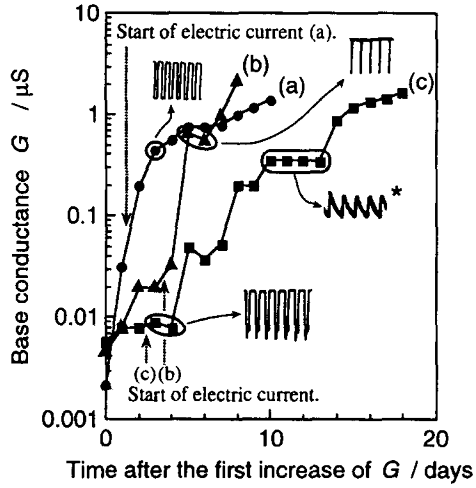

Fig. 6. Change in the base membrane conductance with time for 3 samples, together with oscillation curves observed at each point. Salt solution separated by the filter is 0.5 M NaCl. Time of the first stepwise increase of G is taken as the origin of the horizontal axis.

did not occur. Consequently, no oscillation could be observed when the salt concentration was, from the beginning of the experiment, kept at 2–3 M. This fact indicates that the membrane is more stable under a condition of high salt concentration. Therefore, in order to make use of this salt concentration effect on the membrane stability, an experiment was started under the condition of 0.5 M NaCl on both sides of the filter. After stepwise increase(s) of G was observed, the solution at both sides of the filter was replaced by 3 M NaCl. Fig. 7 is a comparison of the change of G for samples with and without replacement. After the replacement, G was kept constant in contrast to the sample without replacement. The increase of G just after the replacement in Fig. 7 is probably due to the difference in electric conductivity of the solution (refer to Sections 3.3.2 and 4.)

After the replacement, by an application of electric current, oscillations with higher stability occurred. Fig. 8 shows changes of G with time plotted together with oscillation curves observed at each point. For sample (a) in Fig. 8, G was kept almost constant for more than 3 weeks before the start of electric current by changing the salt concentration to 3 M. When the application of electric current was started, an oscillation of downward pulse started and continued for 4 weeks. During these 4 weeks, G was kept at an almost constant level. After that, G decreased spontaneously and the oscillations disappeared on the 60th day. Then, the salt concentration was changed again to 0.5 M, aiming at an appropriate increase (recovery) of G. As a consequence of this operation, G recovered gradually its higher level during 2 weeks of 0.5 M salt concentration, and, after changing the salt concentration again to 3 M, a stable oscillation of the sawtooth type occurred by applying an electric current. Also in case of the sample (b), G was kept at an almost constant level and oscillation(s) continued for a long period. The interval from the first increase of G to the replacement of salt solution for sample (b) was made longer than that for sample (a) in Fig. 8, and this procedure affected the level of G and oscillation characteristics. This point will be discussed in Section 3.3.3.

In conclusion, lifetime of the membrane and that of each oscillation became much longer by changing the salt solution to 3 M. However, it must be noted that the period of oscillations was generally longer comparing with the oscillations previously reported for DOPH impregnated membrane [8]. Therefore, when estimating the 'stability of an oscillation' by the total number (times) of potential changes in a lifetime of an oscillation, 1000–20000 in our case, the stability obtained is around the same order of magnitude as those reported for DOPH, where oscillation with periods of 7 s typically continued for 2–3 hours.

3.3.2. Stepwise increase of membrane conductance

The amplitude of the stepwise increase of G before oscillation, G_s, increased with the salt concen-

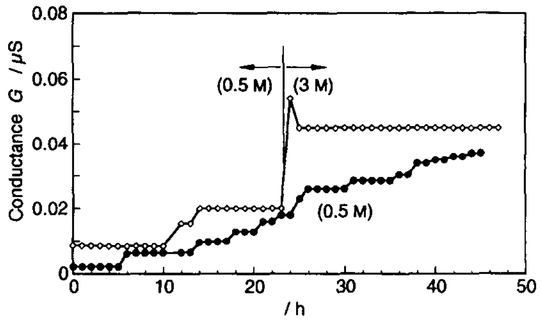

Fig. 7. Change in G with time for samples with and without the replacement of the 0.5 M salt solution by that of 3 M.

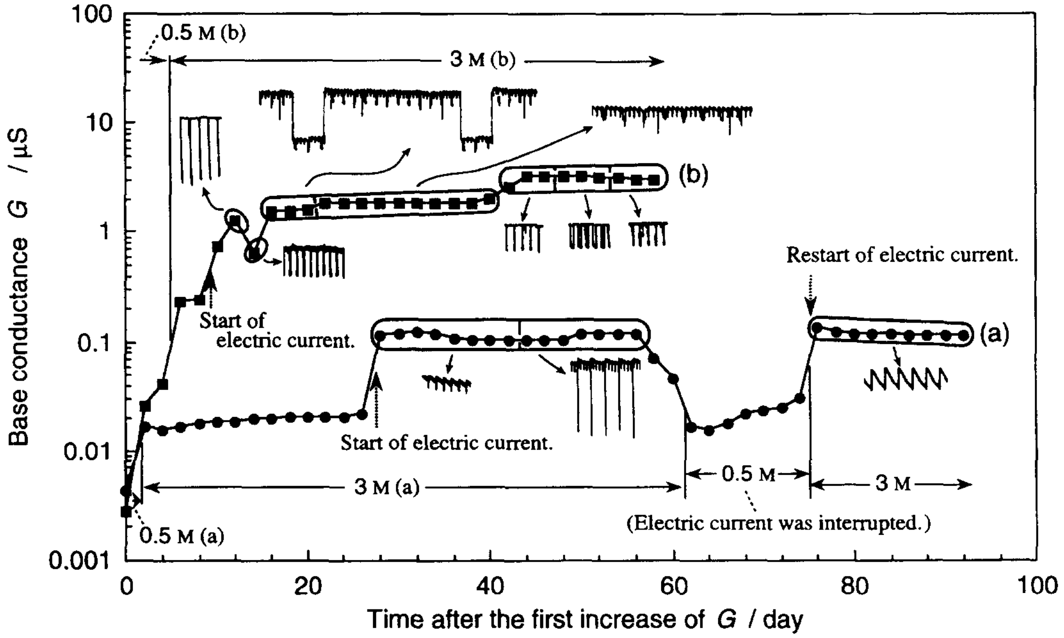

Fig. 8. Change in the base membrane conductance with time for 2 samples, together with observed oscillation curves. The concentration of salt solution was changed from 0.5 M to 3 M before applying electric current, as indicated in the figure. For sample (a), the concentration was changed once again to 0.5 M from 62th to 74th day to recover the appropriate level of conductance. The time of the first stepwise increase of G is taken as the origin of the horizontal axis. The interval from the first increase of G to the change of salt concentration for sample (b) was longer than that for sample (a) (see Section 3.3.3).

tration. Fig. 9 shows relationships between normalized G_s, $G_{s\mathrm{norm}} = (\sigma_{0.5\ \mathrm{M}}/\sigma)G_s$, and salt concentration, where $\sigma_{0.5\ \mathrm{M}}$ denotes electric conductivity of 0.5 M NaCl and σ denotes that of NaCl solution at each concentration. Normalization is done so as to cancel the difference in electric conductivity of the salt solution at each concentration. After the normalization, $G_{s\mathrm{norm}}$ had little dependence on the salt concentration. Stepwise increase of G was not usually observed at 3 M as described above, but even after changing the salt concentration from 0.5 M to 3 M, a stepwise decrease of G followed by a stepwise increase was sometimes observed. Those observations are treated as data at 3 M in Fig. 9.

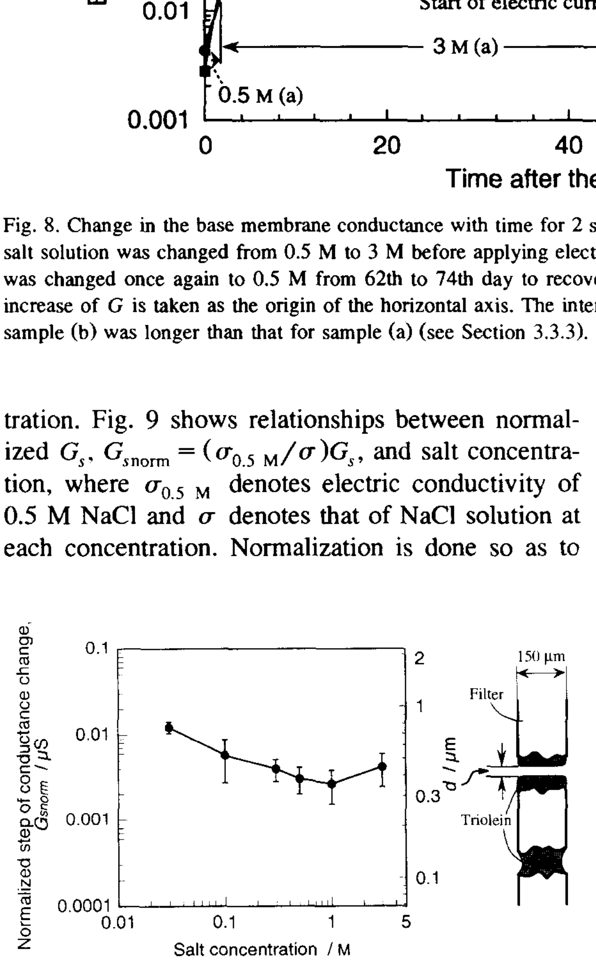

Fig. 9. Relationships between the normalized amplitude of the stepwise increase of membrane conductance, $G_{s\mathrm{norm}}$, and salt concentration of the aqueous salt solution separated by the filter. As for the scale and illustration of the right-hand side, see Section 4.

3.3.3. Control of oscillation characteristics

As described in Sections 3.1 and 3.3.1, stepwise increase of G occurred successively when the salt concentration was kept below 1 M, and G was stabilized after changing the salt concentration to 3 M. The number of the steps before the change of the salt concentration affected the oscillation characteristics, and there was a tendency that mixture of several oscillations was observed more often when the membrane was stabilized, by changing the salt concentration to 3 M, after more steps of increase of G. For instance, in Fig. 8, sample (b) was stabilized after more steps of increase of G than sample (a), and a mixture of 3 or 4 oscillations (2 downward pulse and 2 bistable, or 2 downward pulse and 1 bistable) was observed between the 16th and 40th day. Moreover, the total number of oscillation curves observed for sample (b) was more than that for sample (a).

Generally, such mixed oscillations seem to be independent of one another, because no synchronous relationship among phases of two or more oscillations was observed and the slope of potential/period relationship was different from one oscillation to another. However, the occurrence of entrainment cannot be denied when periods of two oscillations have a similar order of magnitude and some other conditions are satisfied [16,18].

4. Discussion

Several models for potential oscillations observed in lipid impregnated membrane have been proposed. Toko et al. proposed a detailed theoretical model for oscillations observed for membrane impregnated with DOPH and presented numerical results [8]. In their model, the oscillations were attributed to repetitions of opening and closing of a hole through the membrane, which occurs as a result of phase transition of DOPH molecules coupled with accumulation and release of salt in a pore. Yoshikawa et al. proposed a qualitative model similar with the above one for oscillations observed for membrane impregnated with sorbitan monooleate, where the opening and closing of a hole was attributed to phase transitions between 'water in oil' and 'oil in water' emulsions [11]. Sugawara et al. directly observed such opening and closing of a pore in single-hole system impregnated with DOPH by a microscope [9]. On the other hand, Kawakubo proposed a model where potential oscillations were explained as a result of autocatalytic adsorption and desorption of ions at the surface of the membrane [19]. Fuchikami et al. modified Kawakubo's model so as to include the counter ions explicitly [20].

All data we obtained can be explained by a similar model as Toko et al.'s and Yoshikawa et al.'s. That is, the generation of the potential oscillation under an application of constant electric current, that is an oscillation of membrane conductance, can be explained as a periodic change of the diameter of a hole through the membrane, which has opened in one of the pores filled with triolein, as schematically illustrated in Fig. 10. Each of the stepwise increases of the membrane conductance that is necessary for the occurrence of the oscillation corresponds to an opening of the hole. Base membrane conductance, G, is attributed to the total electric conductivity of the salt solution in all the holes opening parallel with each other, and the conductance amplitude, ΔG, corresponds to the rhythmic variation of the cross sectional area of a hole. The fact that the temperature dependence of both G and ΔG was parallel to that of the salt solution supports this explanation.

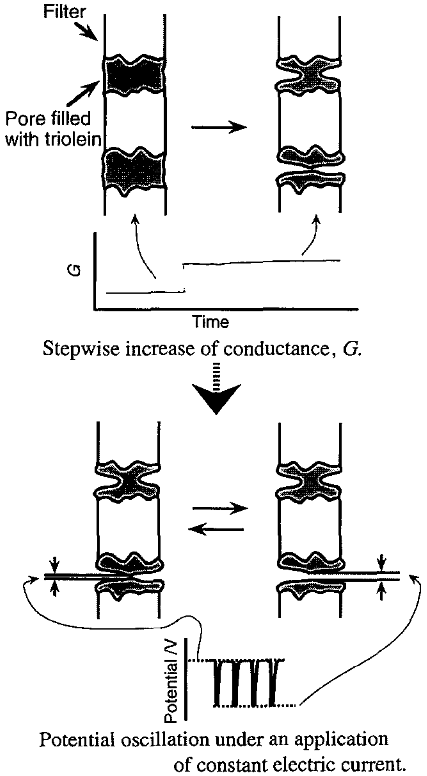

Fig. 10. A schematic illustration of the stepwise increase of membrane conductance and the oscillation of electric potential, appropriate to explain the experimental data.

The experimental results of salt concentration effects imply that the membrane becomes 'harder' and a new hole becomes more difficult to open as the salt concentration is raised. The results shown in Section 3.3.2, where G_{snorm} had little dependence on the salt concentration, indicates that the diameter of the hole was independent on the salt concentration. The vertical axis of the right-hand side of Fig. 9 represents diameter of a hole, assuming that it opened with a constant diameter through the membrane by each step of the increase of G. The diameter is estimated to be 0.39 ± 0.07 μm at for instance a 0.5 M salt concentration, which is a reasonable value compared with the pore size of the filter. However, it must be noted that this assumption is rather simple considering the complicated shape of pores in the Millipore filter, and the diameter of a hole may be smaller than the estimation [16].

The dependence of characteristics of oscillation curves on the number of the stepwise increase of G, shown in Section 3.3.3, can also be explained by the model, where each step is considered to correspond to an opening of one hole. When the salt concentration is changed to 3 M after more steps of increase of G have occurred under a certain lower salt concentration, the number of holes, each of which has a possibility to participate in the oscillation, is increased more. Consequently, the number of oscillations observed is increased and a mixture of several types of oscillations can be observed more often.

The mechanism to cause the open of a hole through triolein in a pore and rhythmical change of diameter of the hole is still unclear. The mechanism may be different from that proposed for the DOPH membrane, because triolein is a non-ionic lipid in contrast with DOPH and experimental results of the salt concentration effect are rather different from those obtained for the DOPH membrane.

The experimental results obtained in this paper show a successful way to stabilize and control the oscillation in a membrane filter impregnated with triolein. After these experiments, further studies to control shape of oscillation curve, improve stabilization of the system, to analyze oscillation curves and to clarify mechanism of the periodic change of the triolein hole induced by electric current are now in progress.

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