CHEMISTRY LETTERS, pp. 1375-1378, 1986.

# Self-Oscillation of Electric Potential of a Porous Membrane Impregnated with Polymer Multi-Bilayer Complexes1)

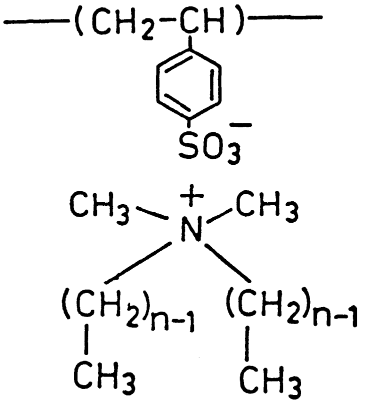
Kiyoshi TOKO,* Naotoshi NAKASHIMA,*† Satoru IIYAMA,††
Kaoru YAMAFUJI, and Toyoki KUNITAKE†
Department of Electronics, Faculty of Engineering
Kyushu University, Fukuoka 812
† Department of Organic Synthesis, Faculty of Engineering
Kyushu University, Fukuoka 812
†† Department of Home Economics, Women's Junior College of
Kinki University, Iizuka 820

A self-sustained oscillation of electric potential across a porous membrane impregnated with polyion complexes of ammonium bilayers and an anionic polymer was observed. The oscillation was associated with the phase transition of immobilized multi-bilayers.

The excitation phenomenon of the biological membrane has attracted much attention in relation to the reception machanism of the biological information and, more broadly, in relation to the formation of dynamic (dissipative) structures in the non-equilibrium system.2,3) During the past few decades, some model membranes giving self-oscillating phenomena have been proposed.3-17) Among these, the oscillation based on the bilayer structure is especially important.8-17) Typical artificial systems are black lipid membranes(BLM)8,12,13) and porous polymer films doped with lipid analogs such as dioleyl phosphate(DOPH).9-11,14-16)

Our current target in this field is to understand the basic principles of the excitation phenomenon of bilayers at the molecular level and to apply them to development of novel excitable bilayers. Black lipid membranes are not very suitable for this purpose because of their mechanical instability and the corresponding difficulty in manipulation. DOPH is chemically unstable and is not readily available as a pure material. It is essential to find new excitable bilayer-forming materials. Synthetic bilayer membranes which were first discovered by Kunitake and Okahata18) appear to be highly suitable materials because of their chemical stability and structural versatility.19) We demonstrate in this communication the self-oscillation of immobilized synthetic bilayer membranes that is associated with the crystal-to-liquid crystal phase transition and occurs under the salt concentration gradient.

Immobilized bilayer membranes were prepared from double-chain ammonium salts ($2C_{16}N^+$ and $2C_{18}N^+$) and sodium poly(styrenesulfonate)($PSS^-$), as described elsewhere.20) These water-insoluble polyion complexes($2C_nN^+$-$PSS^-$) maintain the fundamental bilayer characteristics. The compositions were determined by elemental analysis. Millipore membranes(Type, VSWP; pore size, 0.025 µm; thickness, 150 µm) were immersed in a $CHCl_3$ solution of the polyion complexes for five minutes, and dried. The amount of the complexes adsorbed was determined by weighing.



$2C_nN^+$-$PSS^-$(n=16,18)

The impregnated Millipore membranes were cut into small pieces and subjected to the DSC measurement(instrument, Seiko Denshi, SSC 560). The phase transition (DSC peak top) occurred at 46 °C(the transition range, 38 - 53 °C) for the $2C_{18}N^+$-$PSS^-$ complex immersed in 1 mM and 100 mM aqueous KCl, and 29 °C(the transition range, 20 - 35 °C) for the $2C_{16}N^+$-$PSS^-$ complex under the same conditions. These temperatures are essentially the same as those of simple aqueous dispersions of these bilayers.[21] These data indicate that the molecular organization of the $2C_nN^+$ portion in Millipore membranes is very similar to that of aqueous bilayers. Multi-bilayer structures are most plausible structures, as illustrated in Fig. 1.

The doped Millipore membrane were conditioned in 1 mM KCl solution overnight and placed between two cells which were equipped with a pair of Ag-AgCl electrodes, and electric potential was measured. The details are given elsewhere.[14-16] The experimental apparatus was immersed in a constant temperature bath(accuracy, ± 0.5 °C).

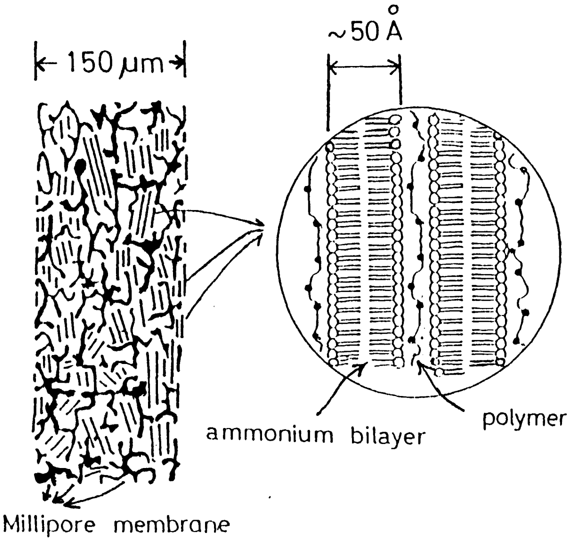


Fig. 1. Schematic illustration of a Millipore membrane impregnated with $2C_nN^+$-$PSS^-$.

The electric potential across 1 mM and 100 mM aqueous KCl which were separated by a Millipore membrane impregnated with the $2C_{18}N^+$-$PSS^-$ (49/51, mol/mol) complex was measured at different temperatures. Oscillation of the electrical potential was not observed at all at 20 °C, 25 °C and 30 °C. The oscillation became clearly noticeable at 35 °C, and was still observable at 55 °C, but not so at 60 °C. A representative example is given in Figure 2a. The period is several seconds and the amplitude is ca. 10 mV. The oscillation is considerably faster than those of the DOPH-Millipore system.[9,10] Similar oscillation was observed for a $2C_{16}N^+$-$PSS^-$ Millipore membrane at 20 - 35 °C and disappeared at temperatures above 40 °C. The temperature regions where the oscillation is observed coincide with the respective phase transition ranges of the

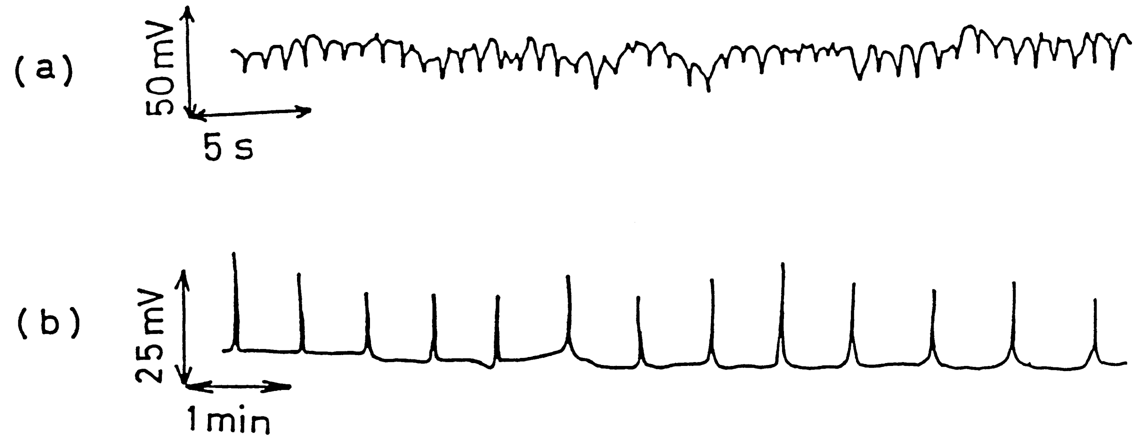


Fig. 2. Self-oscillation of electric potential in Millipore membrane impregnated with $2C_{18}N^+$-$PSS^-$ (0.63 mg/cm$^2$). a:35 °C, b:50 °C

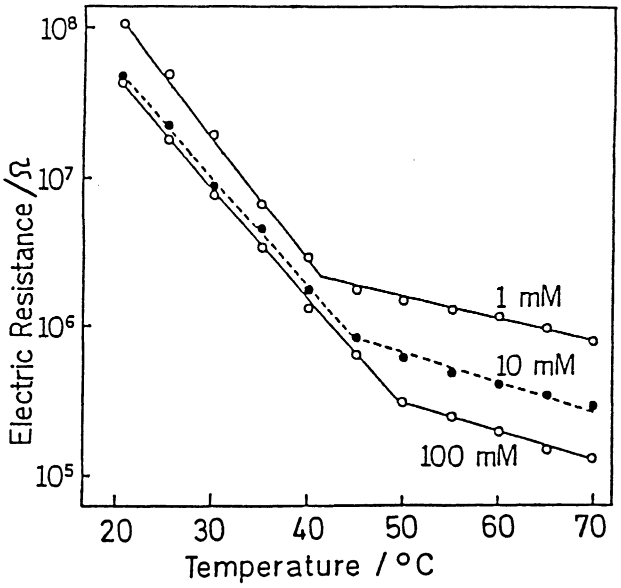


Fig. 3. Temperature dependence of electric resistance of $2C_{18}N^+$-$PSS^-$ (0.31 mg/cm$^2$) doped in Millipore membranes. KCl concentration is given for each curve.

multi-bilayer. This strongly suggested that the self-oscillation is related to the coexistence of the crystalline and liquid-crystalline phases in the phase transition region. Antonov[13)] has also shown the importance of phase transition phenomena for an appearance of current fluctuation under applied voltage in BLM system. In some cases, we observed self-oscillation with much lower frequencies (ca. 0.03 Hz) and with an amplitude of about 15 mV (Fig. 2b). At present, we do not have a technique to selectively induce one type of the oscillations. In fact, a similar situation is reported for porous membranes doped with monoolein.[7)]

The self-oscillation of the membrane electric potential is usually interpreted on the basis of intermittent salt flow. Figure 3 illustrates temperature dependence of the electric resistance of the $2C_{18}N^{+}$-$PSS^{-}$ impregnated membranes which are placed between equimolar KCl solutions. The electric resistance was estimated from the electric voltage across the membrane by imposing a week electric dc current of 0.01 uA on the membrane. The semi-logarithmic plots are constructed from two linear portions. The electric resistane is high at temperatures below $T_c$, and the breaks which coincides with $T_c$ of the corresponding aqueous bilayer are enhanced with increasing KCl concentrations: ca, 44 °C, 47 °C, 49 °C and 55 °C at 1 mM, 10 mM, 100 mM and 300 mM(data not shown), respectively. These results suggest that the physical state of the impregnated multi-bilayer at the two surfaces is made different due to contact with aqueous KCl of different concentrations. The salt effect would be particularly large in the phase transition region where the two phases coexist, and this may lead to the intermittent salt flow.

In conclusion, the self-sustained oscillation of electric potential, i.e., a typical, non-linear and non-equilibrium phenomenon, arised from the coupling of salt flow and the membrane phase transition. The present finding is the first example of self-oscillation of physically and chemically stable synthetic multi-bilayers. The use of the synthetic bilayer membranes will open the door to the design of novel excitable bilayers.

## References

1) Contribution No. 826 from Department of Organic Synthesis.
2) G. Nicolis and I. Prigogine, "Self-Organization in Non-Equilibrium Systems," Wiley Interscience, New York (1977).
3) T. Teorell, J. Gen. Physiol., 42, 831 (1959).
4) V. E. Shashoua, Nature, 215, 846 (1967) ; Faraday Symp. Chem. Soc., 9, 174 (1975).
5) M. Dupeyrat and E. Nakache, Bioelectrochem. Bioenerg., 5, 134 (1978).
6) K. Yoshikawa and Y. Matsubara, J. Am. Chem. Soc., 105, 5967 (1983); 106, 4423 (1984).
7) K. Yoshikawa, K. Sakabe, Y. Matsubara, and T. Ota, Biophys. Chem., 20, 107 (1984).
8) P. Mueller and D. O. Rudin, Nature, 603-604 (1967); 217, 713 (1968).
9) Y. Kobatake, I. Inoue, and T. Ueda, Adv. Biophys., 7, 43 (1975).
10) Y. Kobatake, Adv. Chem. Phys., 29, 319 (1975).
11) J. Arisawa and T. Furukawa, J. Membr. Sci., 2, 303 (1977).
12) A. M. Monnier, J. Memb. Sci., 2, 49 (1977).
13) V. F. Antonov, V. V. Petrov, A. A. Molnar, D. A. Predvoditelev, and A. S. Ivanov, Nature, 283, 585 (1980).
14) K. Toko, J. Nitta, and K. Yamafuji, J. Phys. Soc. Jpn., 50, 1343 (1981).
15) K. Toko, K. Ryu, S. Ezaki, and K. Yamafuji, J. Phys. Soc. Jpn., 51, 3398 (1982).
16) K. Toko, M. Tsukiji, S. Ezaki, and K. Yamafuji, Biophys. Chem., 20, 394 (1984).
17) K. Yoshikawa, T. Omochi, T. Ishii, Y. Kuroda, and K. Iriyama, Biochem. Biophys. Res. Commun., 1985, 740.
18) T. Kunitake and Y. Okahata, J. Am. Chem. Soc., 97, 3860 (1977).
19) See for a recent summary: T. Kunitake, N. Kimizuka, N. Higashi, and N. Nakashima, J. Am. Chem. Soc., 106, 1978 (1984).
20) T. Kunitake, A. Tsuge, and N. Nakashima, Chem. Lett., 1984, 1783.
21) Y. Okahata, R. Ando, and T. Kunitake, Ber. Bunsenges. Phys. Chem., 85, 789 (1981).

(Received May 8, 1986)