Surfactant layer liquid membrane as a model for neuronal excitation

Amlan Kumar Das
Department of Chemistry, Sikkim Manipal Institute of Technology, Majitar, Rangpo, Sikkim (East) 737132, India
E-mail: amlan_snigdha@rediffmail.com

Abstract. Bio-oscillation or biorhythms is one of the most important properties of living organism for maintaining life. There have been many studies on electrical phenomena associated with excitation or oscillation of biological membranes. Most of these studies have been carried out with aim of increasing our understanding of the mechanism of oscillations in bio membranes. In the present study we report a new membrane bilayer system generated by the surfactants, which is bipolar in nature and shows electrical excitability in the absence of any channel former. This new surfactant bipolar liquid membrane shows the promise of being a model for neuronal excitation.

1. Introduction
Several attempts [1-6] have been made to generate model systems for neuronal and other cell membranes. The early experiments of Teorell [2] and of Mears & page [5] continue to evoke interest from electrophysiological point of view in addition to fundamental aspects of instabilities in the non-equilibrium regime.

Muller and Rudin [4] showed that lipid bilayers were not electrically excitable unless channel forming proteinaceous substances were incorporated in them. The significant conclusion from Mueller and Rudin’s work was that excitability was associated with the membrane alone and there was no need to invoke the detailed structure of the cell. This finding motivated several groups to undertake investigation on artificial membrane systems with a view to understanding the possible sources of excitability [7].

In an attempt to mimic the spike potentials of neuronal membranes Shashoua [8,9] experimented with a polyelectrolyte bipolar membrane system prepared by layering a poly-cationic phase onto a poly-anionic phase and separating aqueous solutions of sodium chloride. On application of external electrical potential difference this membrane system showed spontaneous spiking jumps in the transmembrane potential analogous to those observed in neuronal membranes. As far as neuronal membrane are concerned one of the primary requirements of their model systems is that they should be polar in nature, and of course their thickness should be as close as possible to the thickness of the plasma membranes. Shashoua’s polyelectrolyte membranes, though very thick, were bipolar in nature.

In the present paper, a new membrane bilayer system generated by the surfactant, which is bipolar in nature and shows electrical excitability in the absence of any channel former, has been reported.
2. Experimental section
The experimental set up used in this study is shown schematically in figure 1 which has been well labelled to make it self-explanatory. It essentially consists of two compartments, A and B, made from Perspex glass and separated by a Sartorious cellulose acetate micro filtration membrane, M (Cat. No. 11107, pore size 0.2 µm).

2.1. Formation of liquid membranes on hydrophobic supporting membranes
Surfactant layer liquid membrane has been made bipolar by taking cationic surfactant, Cetyl pyridinium chloride (CPCL) in compartment B & anionic surfactant, Sodium lauryl sulphate (NaLS) in compartment A of concentration equal to their critical micelle concentrations (CMC). Both aqueous phase in either side of the membrane containing varying concentration KCl & NaCl, which is more or less, corresponds to the concentrations of the salts inside and outside the axons. On applying an electrical potential differences from an electronically operated electrophoresis power supply (Systronics India Model 610) across the platinum electrodes C and D placed either side of the membrane, the transmembrane potential was found to oscillate with time which was monitored using the Ag/AgCl electrodes E and F connected to an x-t recorder (Digital Electronics Model Omniscribe, series 5000).

![Figure 1. Schematic representation of the cell: M, supporting membrane.](image)

2.2. Experiments for confirmation of liquid membranes formation on supporting membranes
Evidence in favor of liquid membrane formation was obtained from electrical resistance data. The concentration of the surfactant (NaLS) was varied up to its CMC(8.272 mM) and beyond in compartment A, keeping the concentration of NaCl in both compartments A and B (figure 1) constant (0.15 M). Known currents (I) were passed using Pt electrodes C and D, and the potential difference (Δφ) across the Ag/AgCl electrodes E and F was measured using a Philips Model PM 2525 multimeter. The values of the electrical resistances were calculated from (I-Δφ) plots at various concentrations of the surfactant. The experiments were repeated taking CPCl in compartment B with varying concentration up to its CMC 0.9 mM and beyond.
3. Results and discussion
In this situation a bilayer of surfactant layer liquid membrane consisting of the molecules of NaLS and CPCL was shown to be formed within the pores of the supporting membranes in accordance with the liquid membrane hypothesis of Kesting et al [10-12].

3.1. Critical micelle concentration & Kesting hypothesis
Surfactants, commonly called detergents, are amphiphatic molecules having distinct hydrophobic and hydrophilic regions. Depending on the chemical structure of their polar head groups, surfactants can be neutral, cationic, anionic or zwitterionic. By a surfactant we mean a species that accumulates at the interface of two phases or substances (one of which may be air) and modifies the properties of the surface. Over a narrow range of concentration there is a sudden transition in almost every measurable property like electrical conductivity, surface tension, light scattering, refractive index etc. that depends upon size or number of particles in aqueous surfactant solution. This transition corresponds to the formation of aggregates of surfactant molecules and is used to define the critical micelle concentration or CMC.

The Kesting hypothesis states that when a surfactant is added to an aqueous phase, the surfactant layer, which forms spontaneously at the interface, acts as a liquid membranes modify transport across the interface. It further states that as the concentration of the surfactant is increased the surfactant layer liquid membrane progressively covers the interface and at the critical micelle concentration it is completely covered. The evidence in favour of formation of the surfactant layer liquid membrane was obtained from the variation of electrical resistance with concentration of the surfactant (figure 2). The data in figure 2 reveal that increasing trend continues up to the CMC of the surfactant beyond which the value of resistance becomes more or less constant. It appears, therefore, that when compartment A is filled with a solution of NaLS and B with solution of CPCl, concentration being equal to their CMCs, two surfactant layers would be formed, one on either side of the supporting membrane. However, formation of a surfactant layer membrane at the interface may be accompanied by the penetration of the surfactants into the pores as single molecule and also as the micelles. Although the CMC value of the aqueous solutions of the surfactant would not be the same as in sodium chloride solutions, the conclusion about the formation of the surfactant layer liquid membrane is not likely to change qualitatively. The surfactant bilayer formed would be bipolar in nature; the face of the surfactant layer in the compartment containing NaLS solution would be negatively charged and that in the compartment containing CPCL solution would be positively charged. The resting membrane potential of the system Ag/AgCl, NaCl (0.15M), CPCl (0.9mM, i.e.CMC)/ cellulose acetate membrane / NaLS (8.27 mM, i.e. CMC) NaCl (0.15 M) AgCl/Ag was found to be 4.98 mV with the compartment containing CPCL as positive.

Figure 2. Variation of electrical resistance with the concentration of the surfactant. Curve a, is for CPCL in compartment B and curve b is for NaLS in compartment A. In each case the NaCl concentration in both compartments is 0.15M.
3.2. Transmembrane potential oscillations on application of external electrical potential differences

On applying electrical potential difference across the membrane, the trans-membrane potential was found to oscillate with time. A few typical traces are reproduced in figures 3 and 4. In each case it was observed that the frequency of the oscillations was initially high which showed a decreasing trend and finally the oscillations ceased. But after some time the oscillations reappeared with the same qualitative trend in the frequency, i.e. initially high, then low and then no oscillations and so on. Experiments were also performed by taking NaCl on one side of the membrane and KCl on the other and also by taking mixtures of NaCl and KCl solutions on the two sides of the membrane. The composition of the mixture was so chosen that it more or less corresponded to the concentrations of the NaCl and KCl inside and outside the axon. In all cases oscillations of trans-membrane potentials were observed (figure 4). In all cases it was observed that unless the externally applied voltage exceeded a certain minimum value, oscillation were not observed. It was also observed that the oscillations ceased if the applied voltage exceeded a certain maximum value. For example, in the experiments with 0.15M NaCl in compartments A and B (figure 3b), oscillations were observed only when the applied voltage was between 1.24 and 1.9 V. In the traces shown in figures 3 and 4 the applied voltage was 1.24 V with compartment containing NaLS as positive. Thus, there exists a threshold value of applied voltage for the occurrence of oscillations. Another necessary requirement for the occurrence of oscillations is the proper polarity of the electrodes while applying the voltage externally; the electrode in the compartment containing NaLS should be positive and that in the compartment containing CPCl should be negative. The observations on the polarity of the electrodes and on the existence of a lower limit (the threshold value) and an upper limit of the applied voltage between which only the oscillations were obtained are similar to those made by Shashoua in his experiments.

![Figure 3](image)

**Figure 3.** Traces of the trans-membrane potential oscillations. In each case compartment A contained NaLS (CMC) and compartment B contained CPCl (CMC). The value of the imposed potential difference is 1.24V, with the compartment containing NaLS as positive. The scale shown is applicable to all traces a-c. Curve a: for the situation when no electrolytes (NaCl or KCl) are added in compartments A and B and for the situation when they are added, the arrow indicates when a few drops of NaCl are added in both compartments. Curve b: 0.15M NaCl in both compartments. Curve c: 0.15M KCl in both compartments.

The mechanism of such oscillations has not yet been fully deciphered; an attempt in these directions should form a valid project for the future. The mechanism of membrane potential...
oscillations invoking phase transition in the case of surfactant/lipid-doped filters has not been corroborated by Kim and Larter [13] in their experiments with filters doped with DOPH and OA. The membrane system investigated in the present study being bipolar is closer to Shashoua’s [8] which had three zones: a positively charged zone, a neutral zone and a negatively charged zone, the neutral zone being the central zone. Katchalsky [14, 15] suggested the following explanation for the electrical potential oscillations in Shashoua’s experiments. Consequently to imposed potential difference, NaCl is accumulated in the central zone, the sodium and chloride ions arriving from opposite sides of the membrane. The increase of the osmotic pressure leads to the flux of solvent into the membrane and to an increase in hydrostatic pressure in it. At the same time the increase of the salt concentration causes the polyelectrolyte molecule to compress, which also increases the pressure. When this increase in pressure exceeds the osmotic pressure, the solvent flux changes its sign and the salt concentration inside the membrane increases even more. A concentration gradient arises and the salt leaves the membrane to flow out after the membrane has attained the maximum concentration. Then follows the relaxation and the membrane returns to its initial state to repeat the process.

**Figure 4.** Traces of the trans-membrane potential oscillations. In each case compartment A contained NaLS (CMC) and compartment B contained CPCL (CMC). The value of the imposed potential difference is 1.24V, with the compartment containing NaLS as positive. The scale shown is applicable to both traces a and b. Curve a: 0.15M NaCl in compartment A and 0.15M KCl in compartment B. Curve b: Mixture of 0.015M NaCl and 0.15M KCl in compartment A and mixture of 0.005 M KCl and 0.15 M NaCl in compartment B.

Although the system studied in this present case is analogous to Shashoua’s, the explanation suggested above cannot be applicable entirely because it is not known whether the surfactant bilayer membrane contracts in the presence of the salt or not. At present a definite mechanism of the phenomena cannot be suggested, but nonetheless, any mechanism should take into account the following facts/observations: (a) the bipolar nature of the membrane; (b) the electro-osmotic flow consequently to the applied electric field should be bi-directional, i.e. the electro-osmotic flow should occur from the compartment containing NaLS to the compartment containing CPCL and from the compartment containing CPCL to the compartment containing NaLS simultaneously. This bi-directional nature of the electro-osmotic flow may also contribute to the generation of instability in the system. (c) There is a lower limit and an upper limit of the applied voltage between which the oscillations occur. (d) In addition to the surfactant layer liquid membranes in series with the supporting membrane, existence of surfactants inside the pores as single molecules and also as micelles cannot be ruled out and hence phenomena related to these should also be considered in the mechanism.
4. Conclusion
In spite of the undeciphered mechanism, the qualitative resemblance of the trends to certain aspects of neuronal membranes suggests that the bipolar surfactant layer liquid membranes reported in this paper can also be good candidates for conducting membrane mimetic experiments related to excitability of neuronal membranes. The convenience with which such bipolar liquid membrane bilayer can be generated and their stability are added advantages of these new systems.

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