**Research article**

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# Is the membrane permeability the primary property for the membrane potential generation?

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The generation of membrane potential is interpreted as the consequence of passive and active transmembrane ion transport governed by the ion channels and pumps embedded in the plasma membrane of a living cell. The membrane can be even quantitatively explicable in the purview of the membrane theory by the use of the Goldman-Hodgkin-Katz equation (GHK eq.). We performed quite simple membrane potential measurements across the surface-modified impermeable polypropylene membrane. Furthermore, the experimental results suggest that membrane permeability to the mobile ions is not necessarily needed for the membrane potential. Our research results indicate that the ionic adsorption mechanism may become a complete alternative to the currently-accepted membrane potential mechanism.

**Keywords:** Membrane potential; Ion adsorption; Permeability; Goldman-Hodgkin-Katz; Association-Induction-Hypothesis**Introduction**

Physiologically, the membrane potential generation is interpreted as the consequence of passive and active transmembrane ion transport governed by the ion channels and pumps embedded in the plasma membrane of a living cell [1-3]. Therefore, the membrane potential is one of the fundamental facets of biological activities. This concept is part of the fundamental biological concept called membrane theory. The membrane potential can even be quantitatively explicable in the purview of the membrane theory by the use of Goldman-Hodgkin-Katz equation (GHK eq.). For example, the typical GHK eq. is given by Eq. 1 when the mobile ions are Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> [1-6]. Although the GHK eq. has been widely used in physiological studies for many decades, it should be applicable even to non-living systems, since the GHK eq. does not contain any quantity available only to the living cell [7,8]. GHK eq. is derived using the Nernst-Planck equation (N-P eq.), and the N-P eq. is backed by thermodynamics which can be absolutely applicable to both living and nonliving systems [5,6,8]. There are no theoretical backgrounds that the GHK eq. is available only to living systems.

$$\phi = -\frac{RT}{F} \ln \frac{P_{Na} [Na^+]_{in} + P_K [K^+]_{in} + P_{Cl} [Cl^-]_{out}}{P_{Na} [Na^+]_{out} + P_K [K^+]_{out} + P_{Cl} [Cl^-]_{in}} \quad (1)$$

$P_i$  of Eq. 1 represents the membrane permeability to mobile ions. If  $P_i$  is zero (an impermeable membrane), Eq. 1 collapses, and the membrane potential across the impermeable membrane is unthinkable. However, the experimental fact is different from this theoretical consideration. One of the authors of this paper (Tamagawa) and his colleague Morita performed the potential measurement across a permeable ion exchange membrane called Selemion AMV (Asahi Glass Co., Ltd. (Tokyo)) which separated two electrolytic aqueous solutions, where the Selemion AMV is a permeable membrane. Tamagawa and Morita fabricated an impermeable Selemion AMV and made the same potential measurement using it in place of a permeable Selemion AMV [9]. They found that the potential across the permeable Selemion AMV and that across the impermeable Selemion AMV were quantitatively the same as each other. Therefore, a question arises inevitably:

Is membrane permeability essential for membrane potential generation?

Several works have discussed the membrane potential without considering the transmembrane ion transport, which is the foundation of membrane theory. For example, Ohki discusses the contribution of fixed charges of the membrane to the membrane potential [10]. However, he never doubted the membrane theory. Yoshikawa et al. observed the spontaneous generation of the oscillatory potential between two electrolytic solutions separated by an artificial membrane [11]. Although their experimental system did not contain any ion transporter, they believe that such a potential was generated by the transmembrane ion transport. So, there have been countless works that could cast doubt on the membrane theory. However, few people had been serious about altering the membrane theory or proposing an alternative theory to the membrane theory. The late G. Ling was exceptional. He had challenged the membrane theory for the entire course of his life [1-3]. In the purview of the Association-Induction Hypothesis (AIH) he proposed, the membrane potential generation is attributed to the spatial fixation of mobile ions due to their adsorption. Although the ion adsorption mechanism may not be fully denied in mainstream physiology, the transmembrane ion transport mechanism in the purview of the membrane theory is still widely accepted as the primary membrane potential generation mechanism. Therefore, the ion adsorption mechanism has been regarded as one of the secondary mechanisms. However, our investigations up to this point even suggest that ion adsorption could be the primary cause

of membrane potential generation. We performed quite simple experiments as described below to see whether the ion adsorption mechanism can be a primary cause of membrane potential generation or not.

### Experiment

Three types of polypropylene (PP) films denoted by PP-1, PP-2, and PP-3, respectively, were prepared. PP-1 was an PP film as was. PP-2 was an AgCl-coated PP film, but only one surface was coated with AgCl. This one-surface- AgCl-coating process was performed by the following procedure: One surface of the PP film was rubbed with sandpaper. The resulting PP film was washed with the running water and underwent the silver mirror reaction. The Ag layer formed on the non-rubbed surface was wiped off using wet paper. Then a tiny amount of bleach was dropped on the Ag layer, turning the Ag layer into the AgCl layer. This PP film was washed gently using deionized water. The resulting film was PP-2 that had an only one AgCl-coated surface. Another PP film, both surfaces of which were coated with AgCl, was fabricated using the same method for the preparation of the PP-2. The resulting PP film had two AgCl-coated surfaces and this AgCl-coated PP film is denoted by PP-3.

The membrane potential across the PP-1 that separates two aqueous KCl solutions was measured using the setup in Figure 1(a) [12,13].  $C_L$  and  $C_R$  represent the concentration of KCl in the L and R phases, respectively. In this measurement,  $C_R$  was kept at  $10^{-4}$  M while  $C_L$  varied from  $10^{-4}$  M. Figure 2 shows the measured potential against  $\log[C_L]$ .

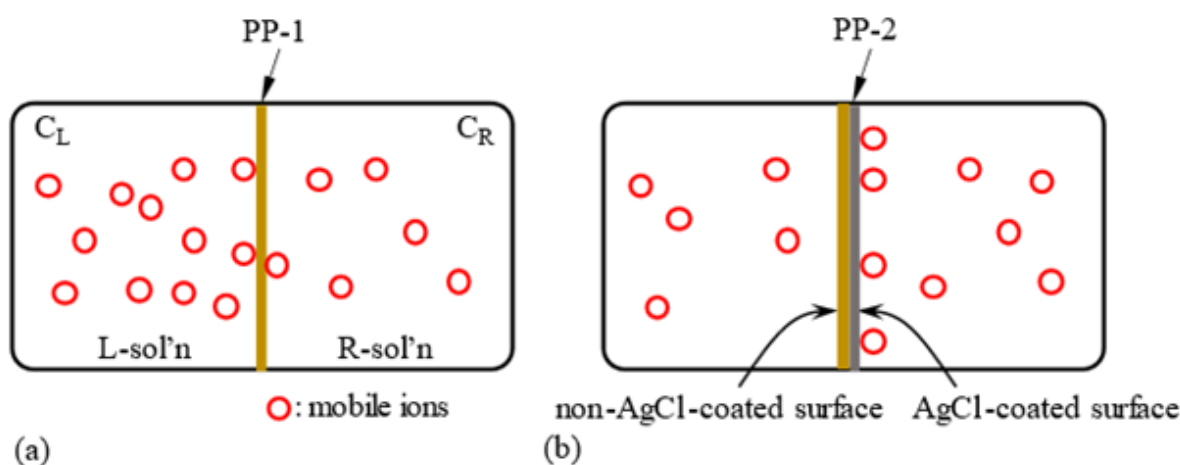


Figure 1: Experimental setup (a) PP-1 is in use (b) PP-2 is in use.

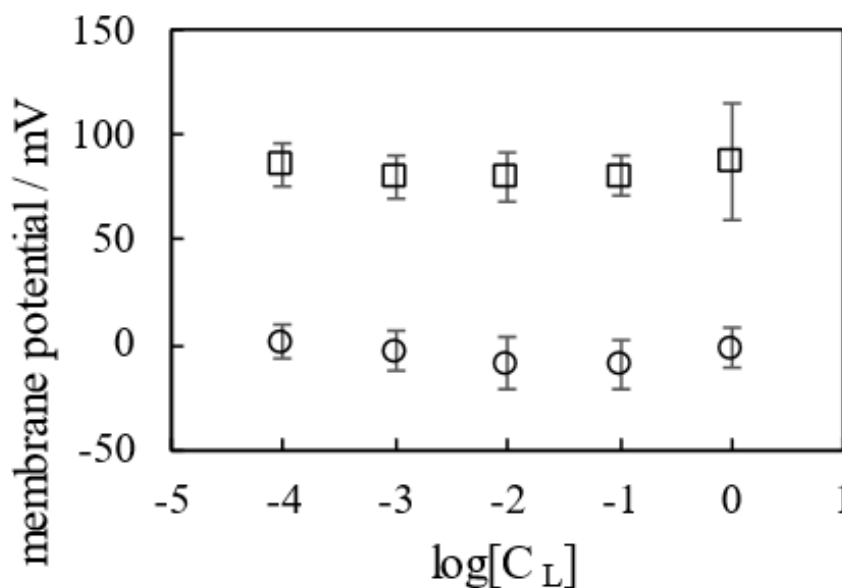
The membrane potential across the PP-1 was indifferent to  $C_L$  and was maintained at 0 mV. GHK eq. for this system is given by Eq. 2. It is possible to find such  $P_K$  and  $P_{Cl}$  such that Eq. 2 can reproduce the zero membrane potentials observed experimentally. Such  $P_K$  and  $P_{Cl}$  are given by Eq. 3.  $P_K$  and  $P_{Cl}$  were estimated so that the GHK eq. can reproduce the experimentally measured membrane potential instead of directly measuring  $P_K$  and  $P_{Cl}$ . This is the common way of finding the numerical value of  $P_i$  of the GHK eq. However, such a process presents us with an awful experience about  $P_i$  determination. That is, PP-1 is an impermeable

membrane. Therefore, its  $P_K$  and  $P_{Cl}$  should be zero as given by Eq. 4. Consequently, the GHK eq. for this experimental system collapses. So, estimating the numerical value of  $P_i$  does not necessarily reflect the actual experimental system.

$$\phi = -\frac{RT}{F} \ln \frac{P_K [K^+]_L + P_{Cl} [Cl^-]_R}{P_K [K^+]_R + P_{Cl} [Cl^-]_L} \tag{2}$$

$$P_K = P_{Cl} \neq 0 \tag{3}$$

$$P_K = P_{Cl} = 0 \tag{4}$$



**Figure 2:** The membrane potential. ○: across the PP-1; □: across the PP-2 where the non-AgCl-coated surface is in contact with L-sol'n while the AgCl-coated surface was in contact with R-sol'n.

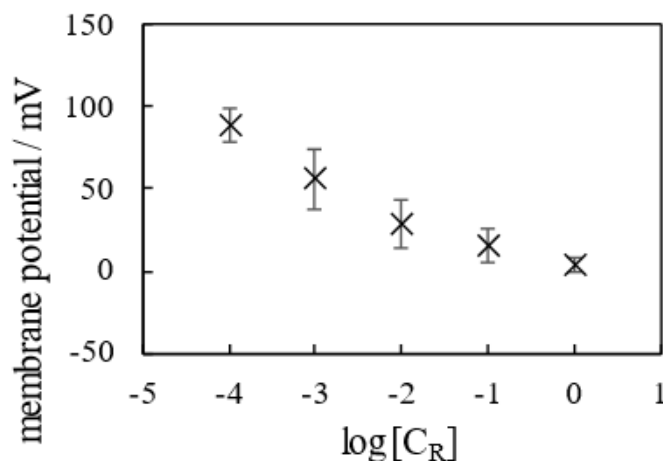
Next, the same membrane potential measurement was performed using PP-2 in place of PP-1 where the non-AgCl-coated surface was in contact with the L-sol'n, while the AgCl-coated surface was in contact with the R-sol'n in this experiment. The membrane potential is shown in Figure 2. This result suggests that the membrane potential on PP-2 was indifferent to  $C_L$  and was maintained constant around 80 mV. This result suggests that the PP-2 behaves as if it were a non-ion-selective membrane in the purview of the membrane theory. We tried to find  $P_K$  and  $P_{Cl}$  that suffice in Eq. 5 (GHK eq.). But mathematically there are no such  $P_K$  and  $P_{Cl}$ . However, a particular potential, 80 mV, was observed regardless of  $C_L$ . There must exist some law governing this membrane potential behavior that is not in line with GHK eq. of the membrane theory.

$$0.08V = -\frac{RT}{F} \ln \frac{P_K [K^+]_L + P_{Cl} [Cl^-]_R}{P_K [K^+]_R + P_{Cl} [Cl^-]_L} \quad (5)$$

Now, we again make measurements of the membrane potential across the PP-2. In this case,  $C_R$  ranged from  $10^{-4}$  M to 1 M and  $C_L$  remained constant at  $10^{-4}$  M, that is, the AgCl-coated surface of PP-2 was in contact with R-sol and its concentration  $C_R$  varied from  $10^{-4}$  M to 1 M. The result is shown in Figure 3 and the membrane potential was found to change according to  $C_R$ . Bear in mind that the horizontal axis of Figure 3 represents the logarithm of  $C_R$  while that of Figure 2 represents the logarithm of  $C_L$ . Although PP-2 was used for both experiments shown in Figure 2 (represented by □) and Figure 3, the membrane potential across PP-2 shown in Figure 3 behaved as if PP-2 were a ion-selective permeable membrane while the membrane potential across PP-2 shown in Figure 2 suggests that PP-2 behaved as if it were a non-ion-selective membrane. However, it is impossible to find the numerical values of  $P_K$  and  $P_{Cl}$  of the GHK eq. that can reproduce the potential in Figure 3.

In summary, the membrane potential across the PP-2 was sometimes quite indifferent to the ion concentration but at other times quite sensitive to the ion concentration as an ion-selective permeable membrane. These potential characteristics cannot be explained by the membrane theory. The characteristics of PP-2 are not consistent from the point of view of current physiology. The currently accepted theory attributes membrane potential generation primarily to the continuous transport of ions across the membrane, but it did not work for this outcome. Alternatively, we would like to suggest that the involvement of ion adsorption in the membrane potential generation and it could be the main cause of the membrane potential generation [1-3,12,13]. See Figure 1(b), which illustrates ion adsorption on the right surface of PP-2.

The left surface of PP-2 is not coated with AgCl, but the right surface is coated with AgCl.  $Cl^-$  tends to adsorb on AgCl [14]. Hence, a certain amount of  $Cl^-$  is localized on the right surface of PP-2. Consequently, the potential on the right surface of PP-2 is different from the bulk phase potential of the R-sol'n. The quantity of adsorbed  $Cl^-$  depends on  $C_R$ . Hence, the membrane potential across the PP-2 shown in Figure 3 exhibits the dependence  $C_R$ . However, the PP-2 left surface is non-AgCl coated. Hence,  $Cl^-$  cannot adsorb on the surface of PP-2. It is interpreted to have no localization  $Cl^-$  regardless of  $C_L$ . Therefore, no dependence of membrane potential  $C_L$  was observed in PP-2, as indicated by Figure 2. We emphasize that ion adsorption can be responsible for the membrane potential generation significantly. This mechanism can also explain the behavior of the membrane potential across the PP-1 shown in Figure 2 (represented by ○). Since neither surface of PP-1 is coated with AgCl, no  $Cl^-$  can adsorb on the surfaces of PP-1. Therefore, the membrane potential across the PP-1 is constant irrespective of  $C_L$ .



**Figure 3:** Membrane potential across the PP-2 where the AgCl-coated surface was in contact with R-sol'n, and C<sub>R</sub> was changed from 10<sup>-4</sup> M through 1 M.

There are two diagrams in Figure 2, one is the membrane potential across PP-1 and the other is that across PP-2. Both exhibit constant potentials regardless of C<sub>L</sub>, but their potential values are different from each other. This is due to the potential difference in R-sol'n. We take a look at the diagrams in Figure 2 under the assumption that the ion adsorption is the primary cause of the membrane potential. The potential across PP-1 is virtually zero and is due to the occurrence of no ion adsorption on PP-1 surfaces. Ion adsorption also did not occur on the non-AgCl coated surface of PP-2 regardless of C<sub>L</sub>. However, Cl<sup>-</sup> adsorption takes place on the AgCl-coated surface of PP-2. The AgCl-coated surface of PP-2 is in contact with the KCl R-sol'n whose concentration is C<sub>R</sub> = 10<sup>-4</sup> (= const.). This Cl<sup>-</sup> adsorption caused some potential. Therefore, the membrane potential on the PP-2 was different from the membrane potential across the PP-1.

All potential profiles are quite consistently explicable by the ion adsorption mechanism. This mechanism was first proposed by G. Ling in the mid-20th century [1-3,15,16]. His theory was fully lost at present, but our research results are in line with his theory. The ion adsorption should not be neglected as the primary cause of the membrane potential.

Considering the ion adsorption phenomenon under electromagnetism, the ion adsorption (and desorption) can be interpreted as the charge annihilation (and creation), and such a process can be further interpreted as the non-zero potential environment. For example, the dissociation of a KCl generates a positive charge K<sup>+</sup> and a negative charge Cl<sup>-</sup>, and the binding between K<sup>+</sup> and Cl<sup>-</sup> results in the annihilation of positive and negative charges. Consequently, the environmental potential is inevitably under the heavy influence of ion adsorption (or desorption) in view of electromagnetism. "Transmembrane ion transport" or "Ion adsorption", which is the primary cause of membrane potential generation? In order to more clearly answer this question, another experiment was performed, as described below.

The potential across PP-3 was measured. We used two types of

PP-3. One has a quite tiny hole at its center and the other one does not have any hole. In order to distinguish the PP-3 with a hole from the PP-3 with no holes, the former one is to be called PP-3H, and the latter one is simply called PP-3 from now on. PP-3H is a permeable membrane and does not have any ion selectivity. Figure 4 shows the experimental setup in which two KCl solutions were separated by PP-3H. C<sub>L</sub> was 10<sup>-4</sup> M KCl and C<sub>R</sub> was a solution of 1 M KCl. The theoretically expected potential,  $\phi_H$ , is given by the GHK eq. of Eq. 6 in purview of the membrane theory where the conditions for this experiment are given by Eqs. 7, 8 and 9. The theoretically expected potential by Eq. 6 turned out to be 0 V. Figure 5 shows the experimentally measured potential in PP-3H. The potential across PP-3H is represented by the dotted line, which indicates the generation of non-zero potential, and this experimental result was in conflict with the theoretically derived  $\phi_H = 0$  V. For comparison, we made the same potential measurement in PP-3 in place of PP-3H using the setup shown in Figure 4. PP-3 is an impermeable membrane. The measured potential profile is shown in Figure 5 by a solid line. This potential is constant about -100 mV.

$$\phi_H = -\frac{RT}{F} \ln \frac{P_K [K^+]_L + P_{Cl} [Cl^-]_R}{P_K [K^+]_R + P_{Cl} [Cl^-]_L} \quad (6)$$

$$[K^+]_L = [Cl^-]_L = 10^{-4} M \quad (7)$$

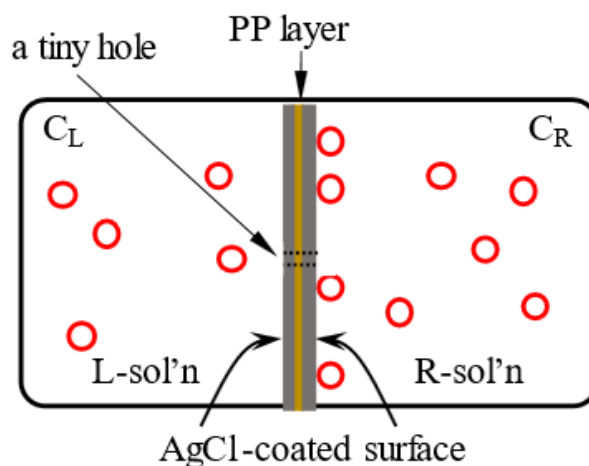
$$[K^+]_R = [Cl^-]_R = 1M \quad (8)$$

$$P_K = P_{Cl} \quad (9)$$

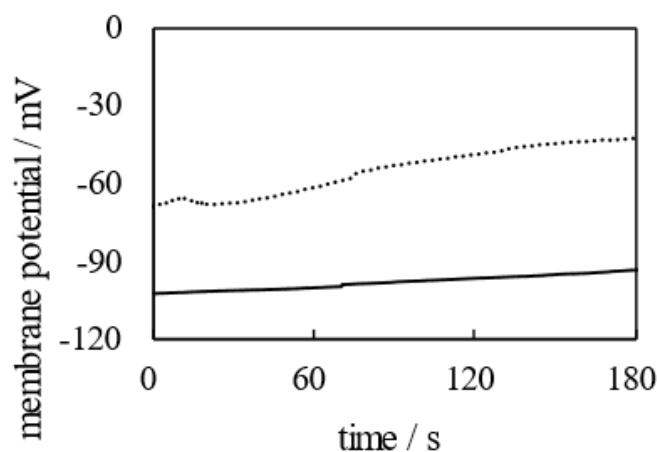
So, the membrane theory cannot reproduce the experimentally measured potential profiles. However, the ion adsorption mechanism can reproduce it. See Figure 6. The adsorbed ions on the PP-3H surfaces can be interpreted as the localized charges. According to electromagnetism, such localized charges inevitably generate the nonzero potential environment. Charge localization can take place whether the membrane is permeable or not. Initially,

the experimental system, in which the PP-3H incorporated, was in the non-equilibrium state  $C_L \neq C_R$  as illustrated in Figure 6(a). Therefore, the quantity of adsorbed ions on the left surface of PP-3H was different from that on the right surface. It caused the non-zero potential in the PP-3H, and corresponds to the potential profile represented by the dotted line in Figure 5.  $C_L$  and  $C_R$  tended to reach the same value  $C_L = C_R$  toward the equilibrium state illustrated in Figure 6(b) due to the diffusion of ions through the tiny hole of the

PP-3H. Due to this tendency, the potential across PP-3H tended to zero (see Figure 5). If the impermeable PP-3 is employed in place of the PP-3H, the adsorbed ion quantity will not vary with time since  $C_L$  and  $C_R$  are kept constant independently due to the occurrence of no ion diffusion across the PP-3. Therefore, the potential change cannot be induced as indicated by the solid line potential profile in Figure 5.



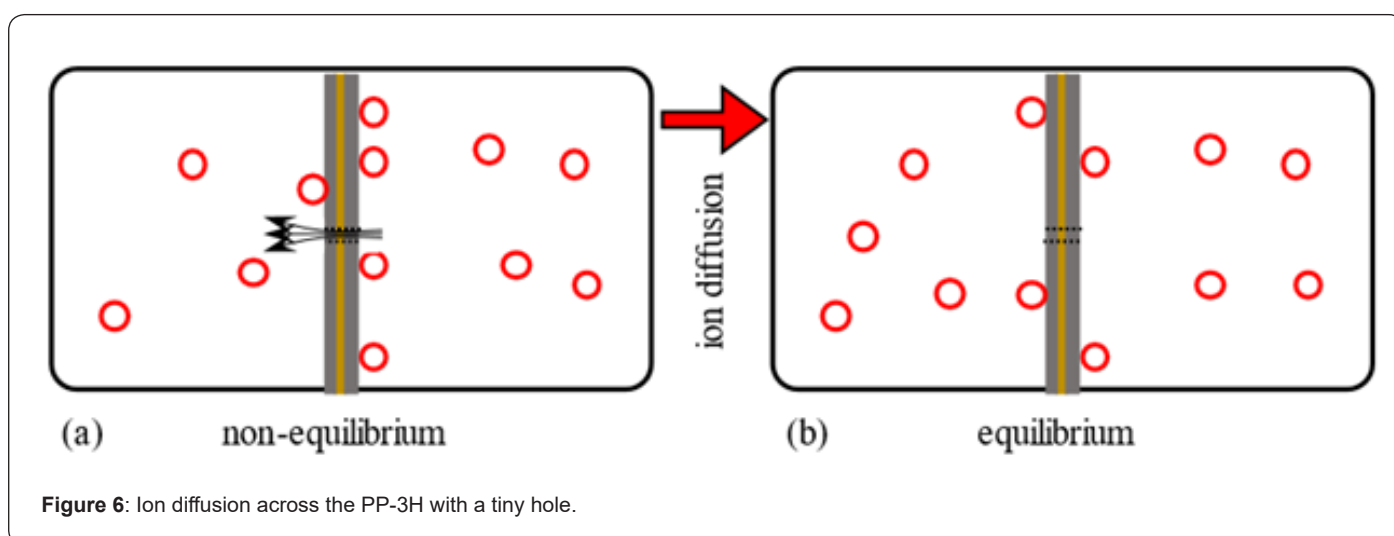
**Figure 4:** Experimental setup for measuring the potential across the PP-3H.



**Figure 5:** Experimental setup for measuring the potential across the PP-3H and the PP-3. Solid line: potential across the PP-3. Dotted line: potential across the PP-3H.

Attention #1: The solid line potential in Figure 5 appears to continue to decrease slightly with time. This is due to the removal of AgCl from the PP-3 surface. AgCl will not stay on the polypropylene surface for a long time, especially in the aqueous solution. Of course, AgCl on the PP-3H also came off with time. However, the potential change across the PP-3H is more significant than across the PP-3. Therefore, the change in the amount of adsorbed ions in PP-3H due to the change in  $C_L$  and  $C_R$  is the predominant factor of the change in the membrane potential in PP-3H.

Attention #2: One may think that the potential across the PP-3H and the potential PP-3 at  $t = 0$  s shown in Figure 5 should be same each other. However, the ion diffusion process across the PP-3H illustrated in Figure 6 began right after pouring the KCl solutions in the experimental setup, resulting in the potential change before stating the potential measurement. This is the reason for the potential difference at  $t = 0$  s between when PP-3H is used and when PP-3 is used.



**Figure 6:** Ion diffusion across the PP-3H with a tiny hole.

Some may say that the artificial cell model dealt with in our research cannot serve as a real living cell model. It is natural to think that the artificial cell model is different from the living cell. Hence, it is quite understandable that our research results are not equivalent to the characteristics of a living cell. However, the fundamental physical, chemical, and biological laws that govern the living systems and those that govern artificial systems should be the same as each other. Physiological phenomena cannot escape the law of electromagnetism. For example, some researchers have raised the question of current physiology by pointing out the significant similarity of action potentials between the living cells and the nonliving microsphere of the proteinoid originally reported by S. Fox [17-20]. Due to their significant similarity, they believe that the action potential is not a consequence of biological activities but is merely a consequence of a physical chemistry-based phenomenon, which is observed in the microspheres. In addition to such an action potential matter, some raised another question: the single channel signal observed by the use of patch clamp technique may not be due to biological activity since the similar biological signal was observable even in the artificial system [21-23]. Our research results suggest that membrane permeability to mobile ions is not necessarily needed for membrane potential generation. The role of ion adsorption in membrane potential generation should not be neglected. Although the ion adsorption mechanism may not become the complete alternative to the currently accepted membrane potential generation mechanism, it is important to reinvestigate the effect of ion adsorption on membrane potential generation for a deeper understanding of membrane potential.

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### Conflict of Interest:

The authors declare that they have no conflict of interest.

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