

Evaluation of a Convenient Method for Determining Membrane Potential

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(Received Aug.25, 1988)

A convenient method for measuring the membrane potential without agar salt-bridge was discussed, considering the accuracy and the merits of this method. It was, consequently, found that measurements by this method are capable of some precision as those by the usual method using agar salt-bridge, and the convenient method is more suitable for measuring the potential change of a membrane with time.

1. Introduction

Normally, the membrane potential is measured by using agar salt-bridge[1]; a sample membrane is set in a cell that consists of two compartments, each filled with a different concentration salt-solution and both compartments are connected to KCl-saturated aqueous solutions via salt-bridge. In both KCl-saturated solutions, electrodes are set to measure the corresponding potential difference. In such measurements, it is not suitable to use bare Ag/AgCl electrodes, because of the need of the correction of Nernst potential to be caused on them and a fear of unfavorable contamination of the electrode surface. Further, such method using agar salt-bridge has the disadvantage that the electrolyte can diffuse out easily from the salt-bridge into the measuring solutions, leading to a change in the concentration of the measuring solutions.

Recently, the membrane potential is often determined by a convenient method using pin-hole type of Ag/AgCl electrodes without agar salt-bridge[2],[3]. In such an electrode, a Ag/AgCl stick hold in a glass cylinder filled with saturated KCl aqueous

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solution is connected with the measuring solution via pin-hole of the glass cylinder. Thus the pin-hole plays a role as micro salt-bridge. However, it has been never reported on the theoretical background and the merits of this convenient method.

In this paper the theoretical descriptions for the convenient method are given and the accuracy and the merits of this method are discussed by comparing the data obtained by this method with those obtained by the usual method with agar salt-bridge.

2. Experimental

Materials: Cationic hydrogel membranes were prepared by copolymerization of acrylamide(AA) and N-trimethylammonium propyl acrylamide(TAPAA) using N,N'-methyl-bis-acrylamide as cross-linking agent[4],[5]. Stock solutions of these two monomers were prepared, so that the total concentration of these monomers became 5 mol/l. Each stock solution was mixed with the same amount of a solution of ammonium peroxide (3.7 g/l) as initiator. This mixture was cast on a glass plate framed with silicone rubber in a circular form and then the casting solution was covered with another plate to prevent the adsorption of oxygen. Polymerization was carried out at 50°C for 5 h.

Measurements of Membrane Potential: Figure 1(a) shows the usual equipment for measuring the membrane potential. A membrane was set in the cell that consists of two compartments: one side, KCl

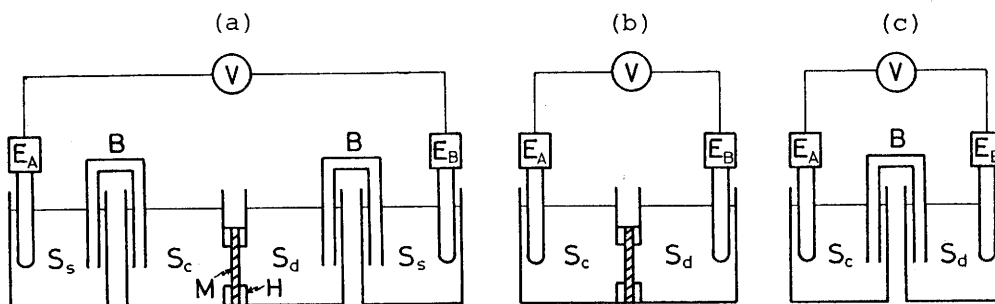


Fig.1 Equipments for measuring the membrane potential: (a) Agar salt-bridge method, (b) Convenient (Direct) method and (c) Asymmetric membrane potential measurement. E_A , E_B ; A_gCl electrode, B; agar salt-bridge, M; sample membrane, V; potentiometer, H; holder(silicone rubber), S_c , S_d , S_s ; KCl aqueous solution(S_c ; $10^{-3}M$, S_d ; $10^{-4}M$, S_s ; sat.)

aqueous solution 1×10^{-3} M; the other side, KCl aqueous solution 1×10^{-4} M. Two Ag/AgCl electrodes (pin-hole type, Horiba Co. Ltd., #2010A) were put in the aqueous KCl saturated solutions, which were connected to both KCl solutions via agar salt-bridges. Both salt-bridges were set as far as possible from the membrane. Potential differences $\Delta\phi_{B/A}$ and $\Delta\phi_{A/B}$ were measured by alternating the inserting position of the electrodes A and B, using a Digital Multimeter TR6843 (Takeda Riken Co. Ltd.,). Temperature was controlled at $25 \pm 0.2^\circ\text{C}$. At the time of each measurement, all electric apparatus except the potential meter were switched off.

Correction of Asymmetric Potential: When an electrode is immersed in a measuring solution, a kind of membrane potential $\phi_{i/o}$ was developed between the inside and the outside of the thin glass membrane of the electrode. The electrode potential between two electrodes observed if they are immersed in a saturated salt-solution, should correspond to the asymmetric potential difference $\Delta\phi_{iB/os} - \Delta\phi_{iA/os}$. Let us suppose the electrode potential difference between both electrodes to be $\phi_{iB/oc} - \phi_{iA/oc}$ and $\phi_{iB/od} - \phi_{iA/od}$, when these electrodes are immersed in unsaturated salt-solutions (concentration: c and d, respectively). These values should be different from the essential asymmetric electric potential $\phi_{iB/os} - \phi_{iA/os}$, because the concentration of electrolyte between the inner and outer solution of the glass membrane of the electrode is not same.

On measuring the membrane potential according to the convenient (direct) method (Fig.1b), the sample membrane was set between two compartments and both compartments were filled with aqueous salt solutions (concentration: c and d), into which the electrodes A and B were then set. The membrane potential $\Delta\phi_{A/B}$ and $\Delta\phi_{B/A}$ measured in this manner are expressed by following relations:

$$\Delta\phi_{B/A} = \phi_{iB/od} - \phi_{iA/oc} + \Delta\phi_m \quad (1)$$

and

$$\Delta\phi_{A/B} = \phi_{iA/od} - \phi_{iB/oc} + \Delta\phi_m \quad (2)$$

where $\Delta\phi_m$ is the membrane potential for the sample membrane. Taking the sum of the Eqns. (1) and (2), the following equation was obtained,

$$\Delta\phi_m = 1/2(\Delta\phi_{B/A} + \Delta\phi_{A/B}) - 1/2[(\phi_{iB/od} - \phi_{iA/oc}) + (\phi_{iA/od} - \phi_{iB/oc})] \quad (3)$$

The second term of the right hand side of Eqn. (3) can be obtained, by measuring the potential difference between the two electrodes A and B exchanging the position of the electrodes in the freshly prepared salt solutions of concentrations c and d, which are connected with a salt bridge (see Fig.1c).

3. Results and discussion

Preliminary-measurements: 1) Electrodes A and B previously immersed in distilled water were inserted in the concentrated and dilute KCl solutions, respectively. $\phi_{iB/od} - \phi_{iA/oc}$ value was measured by inserting a salt bridge intermittently until the value reached to the equilibrium (ca. 5 min). 2) A set of electrodes A and B previously immersed in a dilute KCl aqueous solution respectively were rinsed well with distilled water and then inserted in the opposite KCl solution, respectively. The potential difference between them was equilibrated within 1 min. 3) One electrode was immersed in a concentrated and another one in a dilute solution, and then the salt bridges were placed into both solutions. The change in the value $\phi_{iB/od} - \phi_{iA/oc}$ was followed with the passage of time. After 10 min the value fluctuated vigorously.

Measurements of Membrane Potential of Cationic Membrane: Conditions for preparation of the cationic membrane and some characteristics of the membranes are given in Table 1. That most of the cationic component was successfully incorporated, was confirmed from the equilibrium adsorption amount of Orange II. a) *Convenient(Direct) Method.* With the cationic membrane No.5, the change of potential difference $\Delta\phi_{B/A}$ between both electrodes A and B with time was followed. The result is given with the solid line in Fig. 2. The run was started at the point when both compartments, divided with a sample membrane were filled with KCl solutions. It is clear, that the Donnan membrane potential was obtained after ca. 5 min. Consequently, in every measurement, it was read 5 min after applying the solutions. Further, the value of $\phi_{iB/od} - \phi_{iA/oc}$ in these cases was -1.90 and -1.97 mV, before and after measurements, respectively.

Table 1 Characteristics of sample membranes.

| Membrane No. | 0 | 1 | 2 | 3 | 4 | 5 |
|---|-----------------------------------|-------|-------|-------|-------|-------|
| | monomer concentration (mmol/ml) | | | | | |
| AA | 4.90 | 4.89 | 4.85 | 4.70 | 4.50 | 4.30 |
| MBAA | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| TMAPAA | 0 | 0.01 | 0.05 | 0.20 | 0.40 | 0.60 |
| Thickness* (mm) | 0.480 | 0.483 | 0.545 | 0.557 | 0.743 | 0.871 |
| Diameter* (mm) | 48 | 48 | 52 | 61 | 70 | 78 |
| Degree of swelling* in volume | 1.00 | 1.01 | 1.33 | 1.88 | 3.30 | 4.80 |
| Cation charge* density $\times 10^3$ (eq/dm ³) | 0 | 4.95 | 1.88 | 5.32 | 6.06 | 6.25 |

* in the water-swollen state

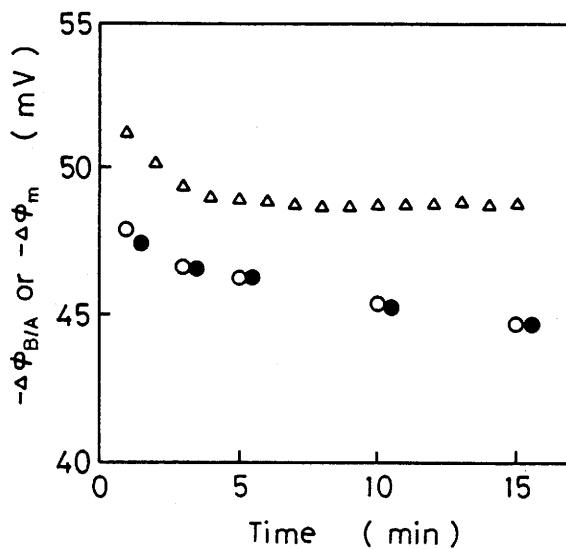


Fig.2 The change of $\Delta\phi_{B/A}$ (measured by direct method) and $\Delta\phi_m$ (measured by agar salt-bridge method) with standing time.

O, ● ; agar salt-bridge method (position of the electrodes was exchanged each other), Δ ; direct method

Next, the values of $\Delta\phi_{B/A}$ and $\Delta\phi_{A/B}$, and the asymmetric potential difference were determined as described above using the same cationic membranes, and then the calculation of the value of $\Delta\phi_m$ was done seven times, to check the reproducibility. The method to obtain the $\Delta\phi_m$ using Eqn.(3) with the correction of the asymmetric potential is named as the convenient method. The results are presented in Table 2. The mean values of $\Delta\phi_{B/A}$ and

Table 2 Observed potential between electrode A and B, and the membrane potential on membrane No.5 by convenient(direct) method.

(mV)

| | $\Delta\phi_{B/A}$ | $\phi_{iB/od} - \phi_{iA/oc}$ | $\Delta\phi_{A/B}$ | $\phi_{iA/od} - \phi_{iB/oc}$ | $\frac{\Delta\phi_{B/A} - \Delta\phi_{A/B}}{2}$ | $\Delta\phi_m$ |
|---|--------------------|-------------------------------|--------------------|-------------------------------|---|----------------|
| 1 | -49.14 | -3.33 | -43.88 | 2.87 | -46.51 | -46.28 |
| 2 | -49.02 | -3.41 | -43.93 | 2.90 | -46.48 | -46.22 |
| 3 | -49.22 | -3.43 | -44.28 | 2.86 | -46.75 | -46.47 |
| 4 | -48.98 | -3.24 | -43.62 | 3.29 | -46.30 | -46.33 |
| 5 | -49.38 | -3.62 | -43.54 | 3.08 | -46.46 | -46.19 |
| 6 | -49.70 | -3.60 | -43.90 | 3.00 | -46.80 | -46.50 |
| 7 | -49.72 | -3.54 | -44.40 | 3.00 | -47.06 | -46.79 |

$\Delta\phi_{A/B}$ without any correction are also given for comparison(direct method). It was concluded that the range of both the values of $(\Delta\phi_{B/A} - \Delta\phi_{A/B})/2$ and $\Delta\phi_m$ were within ± 0.4 mV and that there is small difference (ca. 0.2 mV) in the potential before and after the correction of asymmetric potential. The $\Delta\phi_m$ value obtained using Eqn.(1) was not in agreement with that using Eqn. (2), where $\Delta\phi_{B/A}$, $\Delta\phi_{A/B}$ and each asymmetric potential were measured independently. The difference was about 1 mV.

b) *Agar Salt-bridge Method.* Also with the cationic membrane No.5 the membrane potential $\Delta\phi'_m$ was obtained by the agar salt-bridge method. The measurements were carried out using the same electrodes A and B used in the convenient method and exchanging both electrodes. Measured values for $\Delta\phi_{B/A}$ and $\Delta\phi_{A/B}$ were corrected with the asymmetric potential difference $\phi_{iB/os} - \phi_{iA/os}$ and $\phi_{iA/os} - \phi_{iB/os}$, respectively, which were measured individually, to obtain the value $(\Delta\phi_m)_{B/A}$ and $(\Delta\phi_m)_{A/B}$. The mean value of $(\Delta\phi_m)_{B/A}$

and $(\Delta\phi_m)_{A/B}$ for the value of $\Delta\phi'_m$ was calculated. The same measurements were done three times. The results are given in Table 3. The range is within 0.1 mV, smaller than that obtained

Table 3 Observed potential between electrode A and B, and the membrane potential on membrane No.5 by agar salt-bridge method. (mV)

| | $\Delta\phi_{B/A}$ | $\Delta\phi_{A/B}$ | $\phi_{iB/os} - \phi_{iA/os}$ | $(\Delta\phi_m)_{B/A}$ | $(\Delta\phi_m)_{A/B}$ | $\Delta\phi'_m$ |
|---|--------------------|--------------------|-------------------------------|------------------------|------------------------|-----------------|
| 1 | -47.25 | -45.00 | -1.11 | -46.14 | -46.11 | -46.13 |
| 2 | -47.34 | -44.90 | -1.17 | -46.17 | -46.07 | -46.12 |
| 3 | -47.44 | -45.04 | -1.20 | -46.24 | -46.24 | -46.24 |

by the convenient method. The absolute values are ca. 0.2 mV smaller (more positive) than those by the convenient method. It is natural that no difference was found between the value of $(\Delta\phi_m)_{A/B}$ and $(\Delta\phi_m)_{B/A}$, differing from the results by the direct method. Therefore, it can be said that, in the agar salt-bridge method, the membrane potential $\Delta\phi'_m$ is obtained by taking the mean values of two different membrane potentials obtained by exchanging the position of the electrodes. It is not necessary to measure the asymmetric potential.

Next, the changing of the membrane potential with time was followed by the agar salt-bridge method (In this instance, the value of the potential was corrected with the asymmetric potential). As shown in Fig. 2, it is clear that the potential value decreases with time, because the concentration difference of salt between two solution becomes smaller, as the repetition of electrode-immersing increases.

From the above experimental fact, it is clear that the direct method has an advantage over the agar salt-bridge method for when measuring the potential over a period of time.

Measurements on Cationic Membranes with Different Charge Density:

For the cationic membranes shown in Table 1, the membrane potential was measured by the direct method. Table 4 shows the value $(\Delta\phi_{B/A} + \Delta\phi_{A/B})/2$ obtained without the correction of asymmetric potential and the membrane potential measured by the convenient and the agar salt-bridge method for some of the membranes.

Table 4 Comparison of the membrane potentials measured by convenient(direct) method and agar salt-bridge method

| Membrane No. | Salt | (mV) | | |
|--------------|------|---|-------------------|-------------------------|
| | | Direct method | Convenient method | Agar salt-bridge method |
| | | $\frac{\Delta\phi_{B/A} + \Delta\phi_{A/B}}{2}$ | $\Delta\phi_m$ | $\Delta\phi_m$ |
| 0 | KCl | 5.57 | 5.71 | 6.58 |
| 1 | KCl | -27.13 | -27.17 | |
| 2 | KCl | -35.96 | -35.71 | |
| 3 | KCl | -44.51 | -44.35 | |
| 4 | KCl | -45.32 | -45.16 | -45.79 |
| 5 | KCl | -46.62 | -46.40 | -46.16 |
| 4 | NaCl | -49.45 | -49.07 | -48.15 |

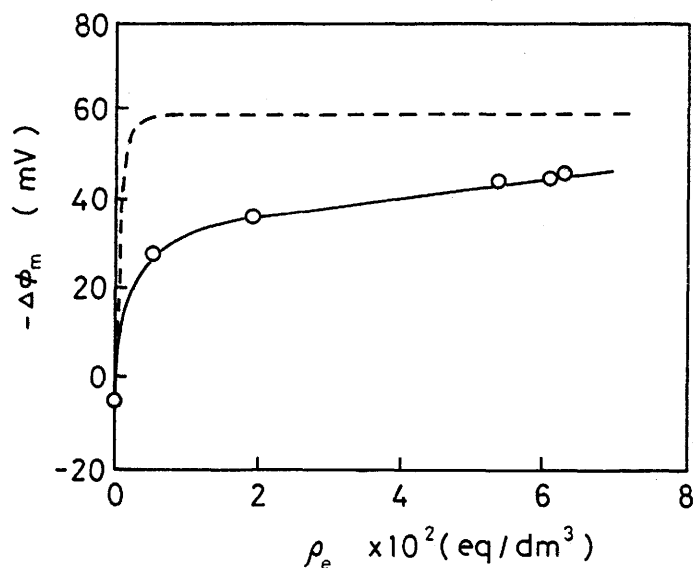


Fig.3 The effect of positive-charge density in the membrane on the membrane potential. The broken line is theoretical curve calculated by the Teorell's equation[6].

In most cases, the values obtained by both the convenient and agar salt-bridge method are slightly shifted in the negative direction, compared with those by the direct method. It can be said that the values obtained by the agar salt-bridge method

are also relatively reliable. The positive values with the membrane No.0 may be due to the presence of a small amount of carboxylic groups caused in part by the hydrolysis of acrylamide groups. Fig.3 show the change of $\Delta\phi_m$ value with the increasing charge density in the membranes.

Measurements Using NaCl Aqueous Solutions: The same measurements were carried out, using NaCl, of which the difference in the transport number between cation and anion is larger than that of KCl. The results are given in the last line. The absolute values are larger than those with NaCl; this is explained considering the additional diffusion potential caused by the larger difference in transport number. From this result, it is clear that the convenient method can be applied for such electrolyte solutions wherein the difference in the transport number is large.

Finally, we would like to refer to the merit of the direct method. Recently, much attention is being devoted to the membrane modification bringing the change of the membrane potential with increasing time. For example, the process of chemical treatment, the adsorption process of solute with high affinity, the following of photochemical response of photosensitive membrane and so on are being investigated. Moreover, it has been reported that the membrane potential is elevated on asymmetrically-charged membranes also in homogeneous electrolyte solution[7]. Therefore, the usual method is unavoidable for the measurement of the membrane potential with treating time in the cases described above, such as chemical changing treatment, asymmetrical adsorption etc.. For such cases, the convenient method developed in this work can become useful.

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