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# Transport numbers in biomembranes from emf measurements

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**Abstract**

Initial membrane potentials of biological periderm and cuticular membranes have been measured with KCl solution using Ag/AgCI electrodes. For the emf measurements, the concentration in both compartment were first brought to equilibrium with 0.01 M KCI solution. After equilibration the concentration in one compartment (inner surface of the membrane) was kept constant as 0.01 M and the concentration in the other compartment was varied between $10^{-4}$ M and 1 M. The procedure was reversed as the concentration in the outer surface of the membrane was fixed and the other side was varied. Transport number of $K^+$ ion corresponding to the each of the two surface layers (homogeneous and heterogeneous) of the membrane was evaluated from the initial time emf measurements. The results obtained were compared with charged polysulfone with polyester supported membrane. 

*Keywords:* Asymmetric potential; Transport number; Periderm membrane; Cuticular membrane; Biomembrane

## 1. Introduction

The description of transport phenomena in biological membranes is still unclear from the physical chemistry viewpoint. The outer and inner surface of periderm and cuticular membranes are different, the inner surface is more homogeneous with its abundance of charged groups. On the other hand, the outer surface appears with a more heterogeneous ultra structure with the epicuticular waxes, which is predominantly uncharged. This asymmetric behavior of cuticular membranes was firstly pointed out by Yamada et al. [1].

The asymmetry potentials of heterogeneous membranes were studied [2–7] when the membranes were in contact with identical electrolyte solutions on both sides. As expected, the asymmetry potentials of biomembranes can be varied with time and vanish eventually. The asymmetry of ion exchange membranes are studied electrochemically by stationary state electromotive force (emf) measurements in well-stirred concentration cells, and by reversing the orientation of the membrane. In general, the interpretation of the emf from such experiments is complex, because the emf is a complicated function of the salt concentration profile in a membrane with asymmetry in the fixed charge distribution, due to sorption of ions and the ratio of diffusion coefficients. The initial time emf method was first developed and investigated by Compan et al. [8–

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10], who applied this electrochemical method to asymmetric polysulfone cellulose acetate, phenolsulfonic acid membrane and polysulfone ultra filtration membranes [4,8,9]. They investigated the initial time experiments, on asymmetric membranes having polyester support layer using small concentration differences. In these studies the transport numbers in the surface layers of the membranes were evaluated from initial time measurements. The emf measurements were immediately taken and transport numbers are evaluated at each face of the membrane.

The emf values of initial time and steady state may predict the interfacial asymmetry by means of the concentration dependence of the mean of the transport numbers for the membrane surfaces. There has always been special interest in the treatment of biological membranes to investigate each face of the membrane. In the present paper, the asymmetry of the two surfaces of the biological membranes was investigated by use of the initial time emf method as well as the steady-state emf method. The intention is to show that emf values might be used to indicate the presence of asymmetry in the biological membranes.

## 2. Experimental

Isolation of periderm and cuticular membranes was carried out using a modification of the previous method [11,12]. The cation exchange membranes, polysulfone with polyester support ($SA_3T$) from Gelman Sciences were used to compare. Basic specifications of the polysulfone membranes is: pore size 0. 45 μm and thickness 152.4 μm as dry, the ion exchange capacity is 0.95 meq/g and supplied in the hydrogen form. A cell made of Borax was used as described previously [14]. The biological membrane was clamped tightly between two compartments of 20 $cm^3$ volumes and the exposed membrane area was 2.05 $cm^2$ (1.33 $cm^2$).

Prior to each experiment, the membrane to be used was immersed for a minimum of 1 day in the solution of the constant concentration (0.01 M KCl), in order to achieve equilibrium. The equilibrated membrane was clamped between two half-cells, which were filled with the solution in the same concentration. The membrane was positioned

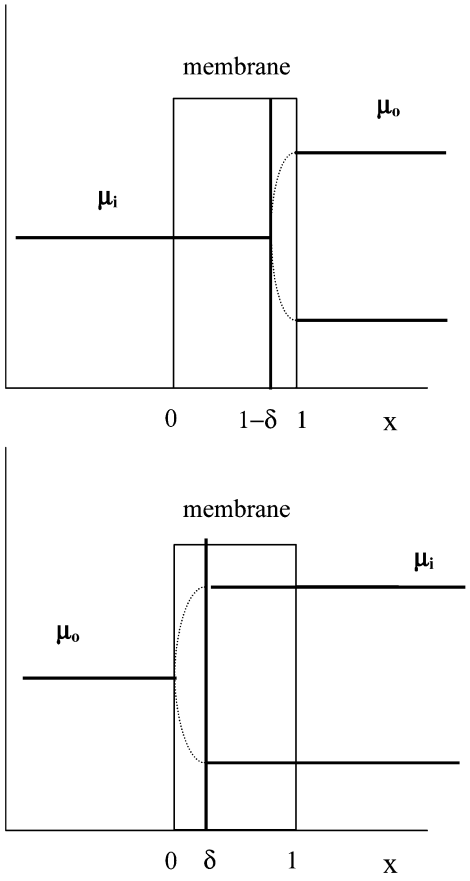


Fig. 1. Salt chemical potential profiles in the biomembranes corresponding of the inner or outer surfaces of the membranes.

in the cells, and both compartments were filled with lower concentration of the solution. The electrodes used were reversible Ag,AgCl electrodes were connected to galvanometer (WPA KED81 DC model) and the system vigorously stirred by magnetic stirrers to minimize the effect of the boundary layers on the potential. In this case, the potential difference between the two equal reference electrodes must be zero. At this point, the membrane potential measurements were performed in the following way, the emf measurements were performed with differences in the concentrations at both sides of the biological membranes, which were always equilibrated with a salt solution concentration equal to 0.01 M KCl and the electrode chambers were filled primarily with this solution until the emf attained a constant value. Always one side of the membrane (homo-

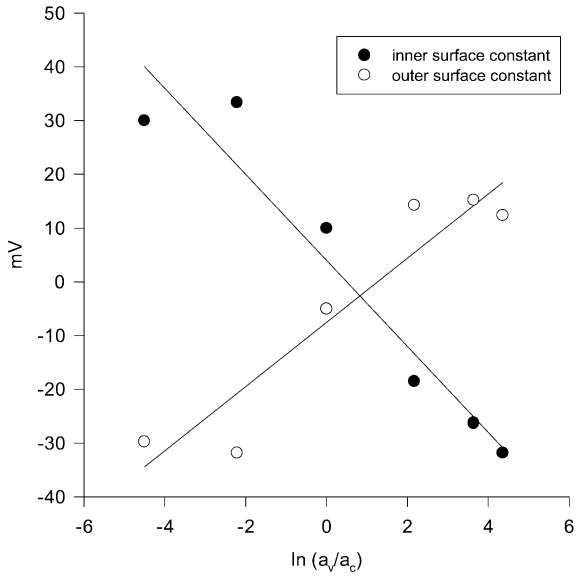


Fig. 2. The initial time emf values for the periderm membrane vs. the natural logarithm of the ratio of the mean activities.

geneous or heterogeneous surfaces) was kept with the equilibrated solution of 0.01 M. Thereafter, the solution in the other side of the cell was withdrawn and replaced immediately with different concentration ranges 0.0001–1.0 M and the emf value is measured immediately with Ag/AgCl electrodes that are already equilibrated in the respective solution. The procedure was repeated with the other faces of the membrane at constant concentration. The experiments are performed at ambient temperature (25 °C).

## 3. Results and discussion

The initial time emf measurements were measured by considering of the surface (homogeneous or heterogeneous) of membrane as constant concentration and the other surface was varied. Principal differences between an inner (i) side time emf and outer (o) side experiments were shown in Fig. 1. Measurement of initial time emf values for periderm, cuticular membranes were measured and polyester supported polysulfone membrane was used to compare. Figs. 2–4 show a plot of the initial time emf values of homogeneous surface (inner surface) constant and heterogeneous surface (outer surface) variable or vice versa for periderm, cuticular and polyester supported polysulfone membranes vs. natural logarithm of the activity gradient ($\ln a_v/a_c$) as a function of concentration gradient. The obtained experimental points of emf values for the membranes with the logarithm of the activity gradients are given remarkably good correlation except the outer surface of cuticular membrane and the obtained regression lines are as follows;

For periderm membranes

$$\Delta\Psi_i(\mathrm{mV}) = -7.997(\ln\ a_v/a_c) + 4.0166 \quad r^2 = 0.925$$

$$\Delta\Psi_o(\mathrm{mV}) = 5.965(\ln\ a_v/a_c) - 7.564 \quad r^2 = 0.889$$

For cuticular membranes

$$\Delta\Psi_i(\mathrm{mV}) = -2.450(\ln\ a_v/a_c) - 0.070 \quad r^2 = 0.854$$

$$\Delta\Psi_o(\mathrm{mV}) = 1.286(\ln\ a_v/a_c) - 9.206 \quad r^2 = 0.582$$

For polyester supported membranes

$$\Delta\Psi_i(\mathrm{mV}) = -4.909(\ln\ a_v/a_c) + 1.957 \quad r^2 = 0.987$$

$$\Delta\Psi_o(\mathrm{mV}) = 4.434(\ln\ a_v/a_c) - 8.750 \quad r^2 = 0.732$$

here, $\Delta\Psi_i$ represents inner surface constant and

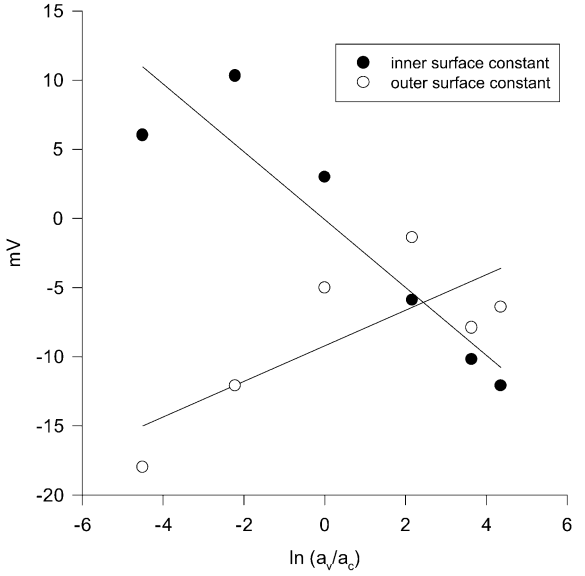


Fig. 3. The initial time emf values for the cuticular membrane vs. the natural logarithm of the ratio of the mean activities.

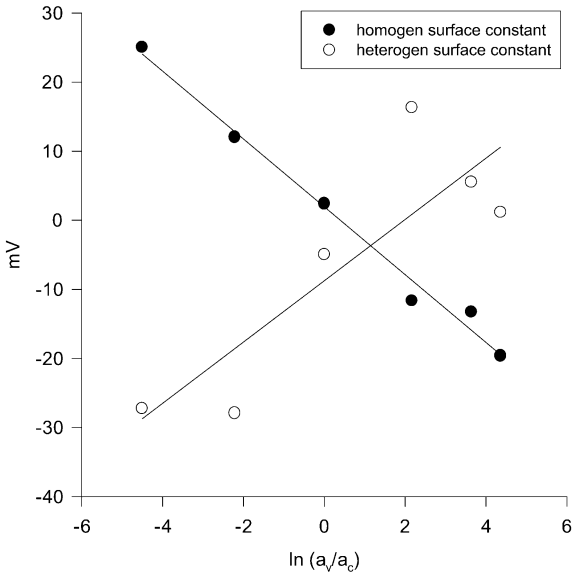


Fig. 4. The initial time emf values for the polyester supported polysulfone vs. the natural logarithm of the ratio of the mean activities.

outer surface variable; and $\Delta\Psi_o$ represents outer surface constant and inner surface variable.

It can be seen from Figs. 2–4 that the extrapolation of curves are not passing through the origin. The experimental points were fitted by regression lines in the range of acceptable correlation coefficient limits except the experiments for cuticular membrane when the concentration in the outer surface was kept constant. However, the initial time of the experiment at both sides of the membrane must have been equal to zero, but the emf values were not obtained zero because of the heterogenity properties of the membranes. When $a_0$ is kept constant and $a_i$ is varied, the slopes of the regression lines are statistically indistinguishable which means that the apparent transport number of $K^+$ ions are equal for both faces. This is due to the large concentration differences between both sides. It has been reported that thin layer of the periderm and cuticular membranes varies depending of their surface layers [13,14] as well as either lower and upper concentration changes.

The asymmetric behavior of periderm and cuticular membranes appears very clear from the experiments data performed with KCl solutions. When compared, the emf values for the two biological membrane faces were quite close to each other. The highest values were obtained when the concentration differences across the membrane were large. It was pointed out that membrane potentials obtained in this way give information about the electro kinetic behavior at the other cuticular membrane surface, and on the other hand the membrane potentials at lower ratios of concentrations are fundamentally Donnan potentials [15–17]. It was also noticed that Donnan effect is more significant at the inner surface region of the cuticular membranes. The inner surfaces of the periderm and cuticular membranes contain mostly protein and lipid molecule, which have a considerable number of dissociable carboxylic groups as well as phenolic groups from which hydrogen ions dissociate. A comparison of the emf values for asymmetric polysulfone with polyester support membrane seems to be close to the values obtained for biological membranes which have inner and outer surface properties. In this manner the initial value emf method seems to work very well.

The initial time EMF values were recorded after 30 or 45 s of contact of the membrane face with the varied new solution. The EMF values measured for biological membranes and the asymmetric polysulfone membranes under initial and stationary state conditions are given in Table 1. For the initial time measurements, it was considered that the transport number as a constant in interval δ because the concentration as shown in Fig. 1 and varies in a thin layer inside the membrane [18]. However, in this study, it was considered the behavior of asymmetric potentials by initial time emf measurements in very large concentration changes. Therefore, the expression derived by Compan et al. [18] was used;

$$\mathrm{EMF}_r = \frac{RT}{F}\tau_2(r)\ln\left(\frac{a_R}{a_L}\right) \quad (\text{‘l side was constant})$$

$$\mathrm{EMF}_r = \frac{RT}{F}\tau_2(l)\ln\left(\frac{a_R}{a_L}\right) \quad (\text{‘r side is constant})$$

The characterization of each surface layer of the membrane was permitted by these expressions [18] which leads to possible determination of the two reduced transport numbers corresponding to the

Table 1
The emf values for the studied membranes under initial and stationary conditions.

| Membrane | $a$ (variable) | $\Delta\Psi_{i,\ in}$ (mV) | $\Delta\Psi_{o,in}$ (mV) | $\Delta\psi_{ist,}$ (mV) | $\Delta\Psi_{o,st}$ (mV) |
|---|---|---|---|---|---|
| Periderm membrane | 0.0000989 | 29.9 | −29.8 | 27.9 | 28.7 |
| | 0.000965 | 33.3 | −31.8 | 34.5 | −32.6 |
| | 0.008896 | 10.0 | −5.2 | 9.4 | −10.1 |
| | 0.077857 | −18.6 | 14.3 | −20.1 | 15.6 |
| | 0.34036 | −26.3 | 15.2 | −28.0 | 16.7 |
| | 0.695 | −31.8 | 12.3 | −29.1 | 14.1 |
| Cuticular membrane | 0.0000989 | 6.0 | −18.0 | 8.5 | −21.5 |
| | 0.000965 | 10.3 | −12.1 | 10.8 | −14.6 |
| | 0.008896 | 3.1 | −5.0 | 3.0 | −7.0 |
| | 0.077857 | −5.9 | −1.4 | −7.3 | −0.5 |
| | 0.34036 | −10.2 | −7.9 | −12.5 | −8.3 |
| | 0.695 | −12.1 | −6.4 | −10.7 | −5.6 |
| Polysulfone membrane | 0.0000989 | 25.0 | −27.3 | 20.1 | −23.6 |
| | 0.000965 | 12.0 | −27.9 | 9.6 | −24.3 |
| | 0.008896 | 2.40 | −5.0 | 1.0 | 1.0 |
| | 0.077857 | −11.7 | 16.3 | −10.0 | 15.0 |
| | 0.34036 | −13.3 | 5.5 | −13.2 | 3.8 |
| | 0.695 | −19.6 | 1.2 | −16.3 | 0.7 |

near interfacial layer of the biological membranes. The mean reduced transport number is calculated by using of the formulas under stationary state conditions, from the following relation [18];

$$\tau_{\text{mean}} \equiv (F/2RT)x\left|\text{EMF} \times (\text{polynomial})/\ln[a_{\text{variable}}/a_{\text{fix}}]\right|$$

Stirring is very important, therefore, the stationary state EMF measurements were performed as constant rpm in both sides in order not to affect the asymmetric potential. The calculated transport numbers of $K^+$ ion from initial time and stationary state emf measurements in the membranes are plotted as a function of the logarithm of the salt concentration (on a logarithmic scale) in Figs. 5–7. It can be seen that the variation of the reduced transport numbers with concentration for both surfaces are very close to each other. It is seen that the concentration dependence is much more pronounced especially the differences is remarkably higher at the lower concentration at the 0.001 M. The curves for the two directions of diffusion are quite close to each other and show similar variation with concentration. The transport number

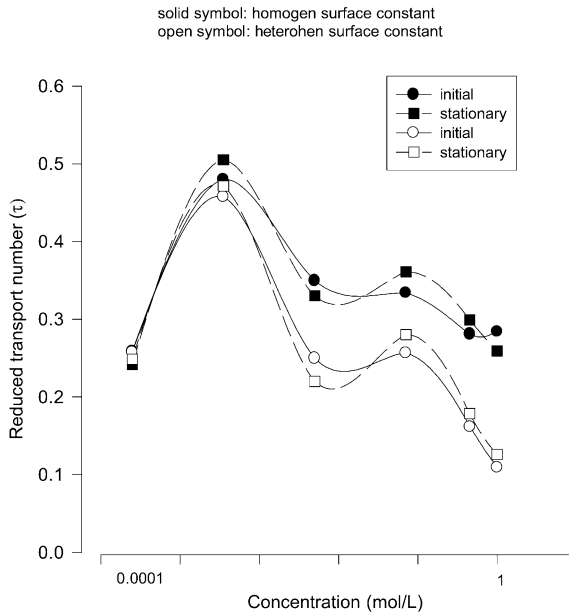


Fig. 5. Reduced initial time transport numbers of periderm membrane for $K^+$ ion for two membrane surfaces as a function of salt concentration.

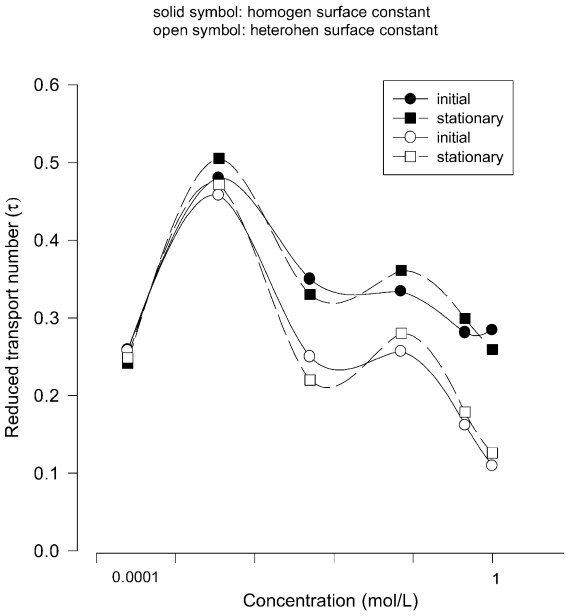


Fig. 6. Reduced initial time transport numbers of cuticular membrane for $K^+$ ion for two membrane surfaces as a function of salt concentration.

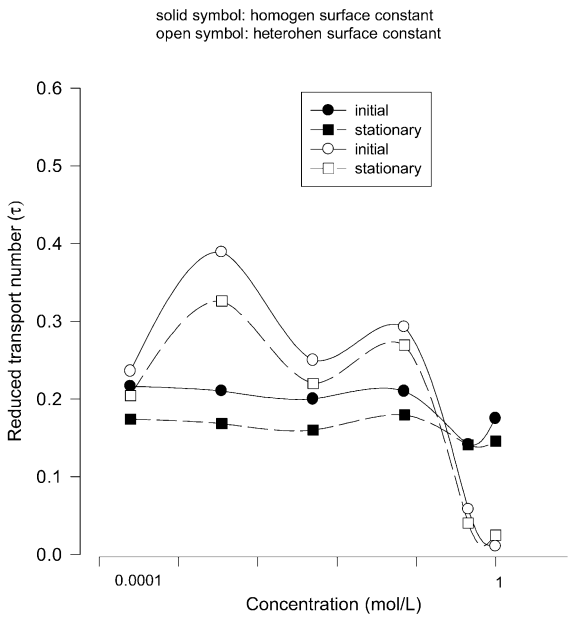


Fig. 7. Reduced initial time transport numbers of polyester supported polysulfone membrane for $K^+$ ion for two membrane surfaces as a function of salt concentration.

of $K^+$ ion in periderm and polysulfone membranes are similar, but is different for cuticular membranes. This may be due to heterogeneity or the functional dissociable groups in cuticles. This is not surprising, since the membrane is heterogeneous depending on the structures of the cuticle.

Alternatively, the initial time emf measurements should be carried out by varying both solutions and also maintain a constant concentration difference between the two external solutions. In this study the concentration difference is too large and the concentration differences was not restricted. Nevertheless, the variation of the transport number with concentration changes for both surfaces of the membranes is approximately linear. The variation of transport numbers with concentration in the surface layer of the membrane turning toward the solution new contact was determined from the regression lines. Therefore, the dependence of transport number to concentration changes either higher or lower from the side might be evaluated.

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