Is the Oil|Water Interface the Simplest and Best Suited Model for Understanding Biomembranes?

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Many studies have been conducted by using the oil (O)|water (W) interface as a simple model for understanding ion transfer (IT) or electron transfer (ET) across biomembranes. In this review, we revisit the usability of the O|W interface as a biomembrane model. For understanding biomembrane IT, the O|W interface is the simplest and best suited model. For example, the standard Gibbs transfer energy of drug ions at the O|W interface is a useful measure for evaluating their membrane permeability in a conventional *in vitro* assay, called PAMPA. However, the O|W interface is not necessarily a good model for understanding biomembrane ET. This is because no net current can be observed with the O|W interface, owing to the ET-coupled proton transfer. In such a case, the self-assembled monolayer (SAM) formed on a metal electrode serves as a better model for understanding biomembrane ET.

Keywords Oil|water interface, ion transfer, electron transfer, biomembrane model, non-Bornian model, self-assembled monolayer

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1 Introduction

Biologically significant processes including respiratory and photosynthetic energy production and neural transmission occur in biomembranes.¹ To understand electron transfer (ET) and ion transfer (IT) processes occurring in such biological systems, various biomembrane models have been proposed, which include oil-in-water emulsion,² planar or supported bilayer lipid membrane,³⁻⁶ self-assembled monolayer (SAM) modified electrode,⁷⁻¹⁰ and the interface between two immiscible

electrolyte solutions (ITIES), the so-called oil|water (O|W) interface.¹¹⁻¹³ A voltammetric technique with the polarized O|W interface, which we call ion-transfer voltammetry (ITV), is very promising for understanding the membrane potential-dependent ET/IT processes in biological systems. Volkov's edited book¹⁴ included many chapters related to biological as well as pharmaceutical applications of the O|W interface.

One of the important applications is the study of the quantitative structure-activity relationship (QSAR). In classical QSAR studies,¹⁵⁻¹⁷ the partition coefficient of compounds between 1-octanol and water ($\log P_{\text{oct}}$) was found to show a linear free-energy relationship (LFER) with biological activities. Since then, $\log P_{\text{oct}}$ has been extensively utilized as the scale of hydrophobicity or membrane permeability of compounds.¹⁸ Since 1992, however, several research groups¹⁹⁻³¹ have used ITV to study the transfer of ionic drugs across O|W interfaces. The standard ion-transfer potential ($\Delta_{\text{O}}^{\text{W}}\phi^{\circ}$) determined by ITV was claimed to be a good measure for the hydrophobicity or biomembrane permeability of ionic drugs and thus for their pharmaceutical activities.

ITV measurements have been also extended to electrochemical elucidation of nerve conduction. Maeda's group³²⁻³⁴ has studied nerve-like, self-sustained potential oscillations of liquid membranes (W1|O|W2 systems) and also their propagation and synchronization in multiple liquid membrane systems. The propagation of the change in membrane potential, such as that from resting potential to action potential, has been studied by Shirai and coworkers,³⁵⁻³⁷ who used biomimetic O|W interfaces or planar bilayer lipid membranes (BLMs) to elucidate the

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potential propagation mechanisms. These studies are expected to lead to advancements in neuroscience.

The technique of ITV can be used to study ET reactions occurring at O|W interfaces. Previously, interfacial ET reactions were studied for several biologically significant redox species, including ascorbic acid,³⁸⁻⁴⁰ FMN,⁴¹ NADH,⁴² and cytochrome *c* (Cyt *c*).⁴³ Kihara's group^{38,41,42} showed that ET reactions involving such biological redox species are accompanied by proton (H⁺) transfer depending on pH and interfacial potential. Equimolar electron and proton transfers occurring at the O|W interface under certain conditions would not give any voltammetric current (for example, see Ref. 42). Additionally, enzyme-catalyzed ET reactions were studied at O|W interfaces by using glucose oxidase^{44,45} and D-fructose dehydrogenase.⁴⁶ In these systems and others,^{39,40,43} it has been suggested that ET reactions should occur not heterogeneously at the O|W interface, but homogeneously in the W phase nearest to the interface (*via* the so-called IT mechanism⁴⁷). Thus, the voltammetric study of ET at the O|W interface would shed light on ET processes in biological systems.

In this review, citing our previous and latest papers, we revisit the validity of the conventional assumption: “*The O|W Interface is the Simplest and Best Suited Model for Understanding Biomembranes*”.

2 IT Processes

Now let us focus our attention on one of the most important applications of ITV, *i.e.*, prediction of the membrane permeability of drugs. As described above, since 1962,¹⁵ log P_{oct} has been extensively utilized as the scale of membrane permeability of compounds. Meanwhile, the values of $\Delta_0^{\text{W}\phi^\circ}$ for ionic drugs were determined by ITV with interfaces between W and 1,2-dichloroethane (DCE),^{19,22-24,27,30,31} nitrobenzene (NB),^{20,21,25,26} *etc.* However, the $\Delta_0^{\text{W}\phi^\circ}$ values determined for some drugs did not show good correlation with log P_{oct} determined in the 1-octanol-W system.^{19,22,24,29,30} As first pointed out by Kontturi and Murtoimäki,¹⁹ this is due to different solvation states of drug molecules in 1-octanol; for example, amine drugs should be solvated differently by forming one or more hydrogen bond(s) or not, depending on the number of protonated amine groups in a drug molecule. Such solvation behavior in 1-octanol seems to differ from that in DCE or NB, and probably from that in the lipid-containing dodecane membrane³¹ being used in a parallel artificial membrane permeation assay (PAMPA). In our view, this demonstrates the superiority of $\Delta_0^{\text{W}\phi^\circ}$ as an index of membrane permeability of drugs.

A couple of decades ago, some methods using human colon carcinoma (Caco-2) cells,^{48,49} Madin-Darby canine kidney (MDCK) cells,^{50,51} *etc.* were developed for evaluating the biomembrane permeability of drugs. Additionally, PAMPA⁵²⁻⁵⁷ using lipid-containing liquid membranes was proposed as an alternative method showing membrane permeability analogous to the above methods using living cells. Among these methods, PAMPA has most frequently been used for a high throughput screening method, because it is less costly, less labor intensive, and more reproducible.

PAMPA is usually performed in a 96-well microtiter plate format, in which the donor (D), membrane (M), and acceptor (A) parts are constructed. In a recent paper,⁵⁸ we reported a digital simulation technique for studying the diffusion of drugs from D- to A-compartment *via* the membrane. This technique enables us to evaluate the permeability coefficient (P_{pampa}) for drugs with different hydrophobicities under different pHs. In a

subsequent paper,³¹ we carried out PAMPA measurements for nine amine drugs to obtain the values of P_{pampa} at different pHs. Applying the simulation method to the analysis of the pH dependence of P_{pampa} , we could determine the “effective” distribution coefficient of each amine drug to the lipid-containing dodecane membrane:

$$K'_{\text{D,M}} = f_n K_{\text{D,M}}, \quad (1)$$

where $K_{\text{D,M}}$ is the “equilibrium” distribution coefficient for the neutral form (D) of the amine drug, which is equilibrated with the protonated form (DH⁺) depending on the acid dissociation index ($\text{p}K_{\text{a}}$) of DH⁺, and where f_n is the fraction of D, being given by

$$f_n = \frac{[\text{D}]}{[\text{D}] + [\text{DH}^+]} = \frac{1}{1 + 10^{\text{p}K_{\text{a}} - \text{pH}}} \quad (2)$$

Using Eq. (1) with Eq. (2) we could determine the values of $K'_{\text{D,M}}$ using the above-determined values of $K'_{\text{D,M}}$ with the literature values⁵¹ of $\text{p}K_{\text{a}}$. It was then found that the log $K_{\text{D,M}}$ values thus obtained by PAMPA showed a LFER with $\Delta_0^{\text{W}\phi^\circ}$ (in V) or log K_{D} , which were determined by ITV with the DCE|W interface:³¹

$$\log K_{\text{D,M}} = -0.833 \Delta_0^{\text{W}\phi^\circ} + 4.95 \quad (n = 9; R^2 = 0.754), \quad (3)$$

$$\log K_{\text{D,M}} = 0.701 \log K_{\text{D}} + 2.89 \quad (n = 9; R^2 = 0.891). \quad (4)$$

These results suggest that log $K_{\text{D,M}}$ (and thus log $K'_{\text{D,M}}$) in PAMPA can be estimated from $\Delta_0^{\text{W}\phi^\circ}$ or log K_{D} at the DCE|W interface. On the other hand, log $K_{\text{D,M}}$ showed a weaker correlation ($R^2 = 0.663$) with log P_{oct} conventionally used as the hydrophobicity scale of compounds in QSAR studies.¹⁸

Here, we would like to stress that $\Delta_0^{\text{W}\phi^\circ}$ can be predicted theoretically by using a recently proposed solvation model called “non-Bornian model.”^{59,60} $\Delta_0^{\text{W}\phi^\circ}$ is related to the standard Gibbs energy of ion transfer at the O|W interface as $\Delta_0^{\text{W}\phi^\circ} = \Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}} / zF$ (z , the charge number including the sign; F , Faraday constant). In the proposed model, local electric fields, E_i ($i = 1, 2, 3 \dots$) on minute surfaces (the area, S_i) of an ion are evaluated by density functional theory (DFT) calculation. Using the calculation result and an experimental data set of $\Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}}$, a multivariate regression analysis can be performed to obtain the semi-empirical equation for $\Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}}$. For the transfer of 26 cations at the DCE|W interface,⁶⁰ we obtained

$$\begin{aligned} \Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}} = & \Delta A \sum S_i + \Delta B^* \sum_{E_i \geq \xi_+} S_i E_i + \Delta C^* \sum_{E_i \geq \xi_+} S_i E_i^2 + \\ & \Delta B_+ \sum_{\xi_+ > E_i > 0} S_i E_i + \Delta C_+ \sum_{\xi_+ > E_i > 0} S_i E_i^2 + \\ & \Delta B_- \sum_{E_i < 0} S_i E_i + \Delta C_- \sum_{E_i < 0} S_i E_i^2, \end{aligned} \quad (5)$$

where seven coefficients (ΔA , ΔB^* , ΔC^* , ΔB_+ , ΔC_+ , ΔB_- , and ΔC_-) were obtained as adjusting parameters in multiple regression analysis, being reported in the previous paper⁶⁰ (where a similar equation was reported for 24 anions). In Eq. (5), an asterisk for ΔB^* or ΔC^* indicates a strongly charged surface with $E_i \geq \xi_+$ (threshold value); such a hydrophilic surface (*e.g.*, on primary to tertiary ammonium groups) coextracts some water molecules to the O phase.⁶¹ This effect is included in the coefficients, ΔB^* and ΔC^* . As seen in Eq. (5), $\Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}}$ is expressed as the sum of the zero-, first-, and second-order terms of E_i . For the positively charged surface with $\xi_+ > E_i > 0$ and the negative charged surface with $E_i < 0$, the first- and second-order terms were divided into two, respectively, because

solvation modes of these surfaces should be different.

Equation (5) thus obtained is available for rather accurate prediction of $\Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}}$ for organic cations. In the DCE|W interface,⁶⁰ the mean absolute error was 1.9 kJ mol⁻¹ for 26 organic cations including nine amine drugs. This error corresponds to the error of ~ 20 mV in $\Delta_O^{\text{W}}\phi^{\circ}$, being comparable with the typical error in ITV measurements (10 mV or so). For the amine drugs, the theoretical values of $\Delta_O^{\text{W}}\phi^{\circ}$ obtained using Eq. (5) showed an excellent linear correlation with the experimental values ($n = 9$; $R^2 = 0.971$).

Thus, the non-Bornian model enables us to predict very accurately the value of $\Delta_O^{\text{W}}\phi^{\circ}$ for the ionic form of a drug. Then, Eq. (3) is available for evaluation of $\log K_{\text{D,M}}$, which can be corrected for the acid-base equilibrium to give $K'_{\text{D,M}}$. Using this value in digital simulation, we can predict the permeation dynamics of the drug in PAMPA, *i.e.*, the value of $\log P_{\text{pampa}}$. Figure 1 shows a satisfactory agreement between the experimental and theoretical values of $\log P_{\text{pampa}}$ (at pH 7.4) for nine amine drugs.³¹ For less hydrophobic drugs with $\log K'_{\text{D,M}} < 2$, $\log P_{\text{pampa}}$ is increased with, $\log K'_{\text{D,M}}$, showing a higher permeability of the drug. For the drugs with $\log K'_{\text{D,M}} > 2$, however, $\log P_{\text{pampa}}$ shows signs of “leveling off,” suggesting that the drugs with hydrophobicity higher than a certain level should show optimal membrane permeability. Nevertheless, as described in previous papers,^{31,58} the amount of a drug transported through the membrane is reduced by increasing $\log K'_{\text{D,M}}$. This is ascribed to the retention of drug molecules in the membrane. Considerably hydrophobic drugs with $\log K'_{\text{D,M}} > 2$, though giving the highest $\log P_{\text{pampa}}$ value, cannot be easily transported through the membrane, from a quantitative viewpoint. In the previous paper,³¹ we suggested that a drug with $K'_{\text{D,M}} \approx 1.5$ should achieve the best permeability, not from a kinetic but also from a quantitative viewpoint.

The above discussion clearly shows that we can utilize the non-Bornian model and digital simulation of PAMPA for making perfect *in silico* prediction of membrane permeability of drugs (illustrated in Fig. 2). This would be useful for labor-saving, shortening the development period, and reducing costs in drug discovery.

As exemplified above by a pharmaceutical application, the O|W interface is a good model for predicting IT processes in biomembranes. Additionally, as reported previously,⁶² the $\Delta G_{\text{tr}}^{\circ, \text{W} \rightarrow \text{O}}$ values determined for IT at the O|W interface were successfully used for discussion of IT processes at BLMs having structural and physicochemical properties more similar to biomembranes.

3 ET Processes

Next, we will discuss the suitability of the O|W interface as a biomembrane model for understanding ET processes *in vivo*. At the O|W interface, ET reactions may take place between

Fig. 1 Plots of the experimental values of $\log P_{\text{pampa}}$ against $\log K'_{\text{D,M}}$ for nine amine drugs (desipramine, imipramine, labetalol, propranolol, alprenolol, acebutolol, nadolol, verapamil, and diltiazem). The solid line represents the theoretical curve obtained by digital simulation. Reproduced with permission from Ref. 31.

Fig. 2 Perfect *in silico* prediction of membrane permeability of drugs.

hydrophobic and hydrophilic redox species respectively added to O and W. The first example was reported by Samec *et al.*,⁶³ who used cyclic voltammetry (CV) to observe a voltammetric wave for the ET between ferrocene (Fc) in NB and $\text{Fe}(\text{CN})_6^{3-}$ in W. However, it was later claimed that the observed current was not due to heterogeneous ET at the O|W interface but due to the IT of Fc^+ that was generated by the oxidation of Fc partially distributed to W.⁶⁴ This mechanism is called “IT mechanism,” in which the ET process occurs in an aqueous reaction layer nearest to the O|W interface. On the other hand, “true” ET due to collision of two redox species at the interface can be realized only by using an extremely hydrophobic redox compound in O, *e.g.*, 5,10,15,20-tetraphenylporphyrin.⁶⁵ In contrast, several ET systems due to IT mechanism have been found using redox compounds including ascorbic acid,^{39,40} Cyt *c*,⁴³ and glucose oxidase.⁴⁵ It should here be noted that such biologically significant redox species undergo ET not at the interface but in the W phase. This would be highly suggestive when considering the behavior of such redox species as Cyt *c* in the mitochondrial respiratory chain.¹ The present finding from voltammetric studies is in line with the general recognition that Cyt *c* undergoes ET in an aquatic environment, *i.e.*, in the thylakoid intermembrane space.

Thus, the O|W interface provides a reaction field for understanding ET behaviors of biological species in a biomembrane-like two-phase system, however its characteristics as the reaction field of ET appear to be rather different from those of biomembranes. This view has come from our recent voltammetric study¹⁰ on the ET between ubiquinone-10 (UQ) and Cyt *c* at a SAM modified electrode, the surface of which seems to have more “biomembrane-like” characteristics than the O|W interface.

In Fig. 3, curve (a) shows a cyclic voltammogram obtained for the UQ-incorporated 1-dodecylmercaptane (DM)-SAM modified gold electrode. The formation of the DM-SAM on a gold disk electrode and the incorporation of UQ therein were performed in a similar manner as reported previously.^{8–10} As seen in the figure, a pair of well-defined cathodic and anodic peaks were observed for the electrode reaction of UQ ($\text{UQ} + 2\text{e}^- + 2\text{H}^+ \rightleftharpoons \text{H}_2\text{UQ}$). These peaks were much widely separated by more than 0.8 V; this suggests a very strong inhibition effect of the SAM on the electrode reaction. As described previously,⁹ this effect is increased with increasing the carbon number of the alkanethiol used for SAM formation, and might be induced not only by the separation of UQ from the gold|SAM interface but also by the inhibition of the ET-coupled proton transfer.

Curve (b) in Fig. 3 shows a cyclic voltammogram for the UQ-incorporated SAM electrode in the presence of Cyt *c*, which was immobilized in advance on the SAM surface by exposure to a phosphate buffer solution (pH 6.8) containing 50 μM Cyt *c*. As shown in the figure, we could observe a well-defined catalytic wave due to the ET between UQ and Cyt *c* in the SAM (*i.e.*, $\text{H}_2\text{UQ} + 2\text{Cyt } c(\text{ox}) \rightarrow \text{UQ} + 2\text{Cyt } c(\text{red})$). The observed catalytic wave did not show a typical sigmoidal curve because of possible saturation of Cyt *c* on the SAM surface, but definitely showed a catalytic effect of UQ on both the cathodic and anodic scans, as shown by diagonal lines in Fig. 3. We would like to add that in the absence of UQ, Cyt *c* gave a weak and ill-defined voltammetric signal at around -0.4 V vs. Ag/AgCl (saturated KCl).¹⁰

Here, it should be stressed that the anodic peak for the re-oxidation of UQ was almost unchanged in amplitude, though the peak potential was somewhat shifted to a more negative value (this is probably because of the facilitation of proton

Fig. 3 Cyclic voltammograms obtained with the UQ-incorporated DM SAM-modified gold electrode in the (a) absence and (b) presence of Cyt *c* immobilized on the SAM.¹⁰ Cyt *c* was immobilized on the SAM electrode surface by exposure to a phosphate buffer solution (pH 6.8) containing 50 μM Cyt *c*. The resultant Cyt *c*-immobilized SAM electrode was immersed in a phosphate buffer solution (pH 6.8), being used as the working electrode. Scan rate: 0.1 V s⁻¹. For further details, see Ref. 10.

transfer induced by a structural disorder of the SAM due to the hydrophobic adsorption of Cyt *c*). This result suggested no significant catalytic activity of UQ for the re-oxidation of Cyt *c*. In order to fathom the cause of such directional or one-way ET, we performed a theoretical analysis using digital simulation with a simple reaction model.⁶⁶ This result showed that the re-oxidation of Cyt *c* should be somewhat thermodynamically unfavorable, but importantly kinetically slow. This slow process is probably originated from spatial separation between the redox species. Similar one-way ET may be occurring in the mitochondrial respiratory chain system to achieve efficient energy production. Probably, one of the significant roles of biomembranes is to prevent redox components from getting too close to each other.

It should also be noted from the theoretical analysis that the formal potentials ($E^{\circ'}$ s) of UQ and Cyt *c* at pH 6.8, being obtained from cyclic voltammograms, are close to those determined in aqueous media. The $E^{\circ'}$ value obtained for the two-electron reaction of UQ at the SAM electrode was +0.17 V vs. SHE at pH 6.8, being close to that reported for UQ adsorbed at a mercury electrode in water (+0.1 V vs. SHE at pH 7).⁶⁷ Similarly, the $E^{\circ'}$ value obtained for Cyt *c* at pH 6.8 was +0.030 V vs. Ag/AgCl (saturated KCl), *i.e.*, +0.227 V vs. SHE, which is close to that determined at the gold electrode in the presence of an effector, 4,4'-bipyridyl (+0.255 V vs. SHE at pH 7). These results suggest that the redox moiety of UQ or the redox center of Cyt *c* should be in a hydrophilic environment, even though these redox species exist in or on the hydrophobic membrane. Since active sites of redox compounds are usually composed of hydrophilic atomic groups (containing oxygen, nitrogen, sulfur, or metal atoms), they may be preferentially hydrated even in hydrophobic membranes.

Thus, the SAM modified electrode is a simple model for providing useful insights into ET processes in biomembranes. But, such is not the case with the O|W interface. Tentatively, we performed CV measurements to assess the capability of ET between UQ and Cyt *c* at the DCE|W interface.⁶⁸ However, no voltammetric wave could be observed for the ET between UQ in DCE and Cyt *c* (red) in W. Further study is needed to clarify its

Fig. 4 Schematic representation of biomembrane IT and ET and the availability of the O|W interface for understanding the respective processes. The O|W interface is a good model for IT, however it is not necessarily a good model for ET, for which the SAM seems to be a better model. For further details, see the text.

cause(s), but we should remember that equimolar electron and proton transfer gives no net current flowing through the O|W interface.⁴² For the SAM modified electrode, however, the ET occurring at the SAM|W interface can be detected as a net current by the base metal electrode, even though ET-coupled proton transfer takes place.

Finally, we would like to add that the solvation environment of redox species in SAM is rather different from that in the bulk O phase of the O|W interface. Certainly, hydrocarbon chains self-assembled on a metal surface form a hydrophobic environment, however incorporation or adsorption therein of redox compounds would produce structural “defects” of the monolayer, through which protons or water molecules may be transported and access the active sites of redox compounds. This seems to result in the above-shown similar redox potentials of UQ or Cyt *c* at the SAM electrode and a naked metal electrode in water. Thus, the hydrocarbon layer of SAM provides a “hydrophilic” environment for redox species, in contrast to the totally hydrophobic O phase of the O|W interface. Accordingly, it is inappropriate to simply consider that the hydrocarbon layer is a thin O phase. Because SAM has an ultrathin thickness (*e.g.*, 1.3 nm for the DM SAM¹⁰), not only protons but also electrons may be transported within the monolayer more easily than for the O|W interface. This situation seems to be analogous to that in biomembranes. The role of the lipid bilayer of biomembranes is not to provide a hydrophobic reaction field for ET, but to immobilize redox components to the membrane by hydrophobic interaction.

4 Conclusions

The answer to the question in the title of this paper depends on the objectives that we want to understand, *i.e.*, biomembrane IT and biomembrane ET.

As illustrated in Fig. 4, in the former case, an ion must pass

through a hydrophobic barrier of the biomembrane. Such a process is analogous to the resolution process in IT at the O|W interface. This leads to a LFER between ΔG°_{tr} (or $\Delta G^\circ_{tr, W \rightarrow O}$) determined with the DCE|W interface and $\log K_{D,M}$ estimated by a conventional permeability assay, PAMPA, with a biomimetic lipid membrane. Because $\Delta G^\circ_{tr, W \rightarrow O}$ and thus ΔG°_{tr} can be accurately evaluated by the non-Bornian solvation model, the combination of this theoretical method and the digital-simulation technique for PAMPA would enable us to perform *in silico* prediction of the membrane permeability of ionic drugs. Thus, one of our answers is that the O|W interface is the simplest and most suitable model for understanding ion permeability across biomembranes.

In biomembrane ET such as in the respiratory chain system, however, ET across a biomembrane is generally coupled with proton transfer, as schematically illustrated in Fig. 4. The proton-transfer coupled ET cannot be observed voltammetrically with the O|W interface. For understanding such ET processes, the O|W interface is not necessarily a good model, whereas the SAM formed on a metal electrode is a better model, enabling us to observe ET as a net current even in the presence of coupled proton transfer. Additionally, the “ultrathin” hydrocarbon layer of SAM provides a “hydrophilic” environment for biological redox species; this seems to be similar to the lipid bilayer of biomembranes (~5 nm thick).

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