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A Strategy for in Silico Prediction of the Membrane Permeability of Drugs

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Abstract

Parallel artificial membrane permeation assay (PAMPA) was performed for nine amine drugs and their permeability coefficient ($\log P_{\mathrm{pampa}}$) was determined at different pH's. The previously developed digital simulation method was successfully used to reproduce the sigmoid-like pH dependence of $\log P_{\mathrm{pampa}}$, and the distribution coefficient ($\log K_{\mathrm{D,M}}$) to the lipid-containing dodecane membrane could be determined for all the amine drugs studied. The thus determined $\log K_{\mathrm{D,M}}$ values showed a linear free-energy relationship with the standard ion-transfer potential ($\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$; R^2 = 0.754) and the distribution coefficient ($\log K_{\mathrm{D}}$; R^2 = 0.891), which were previously determined by ion-transfer voltammetry with the 1,2-dichloroethane|water interface. On the other side, we can predict $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$ very accurately by a previously proposed solvation model called the "non-Bornian" model. These findings suggest a possible strategy for perfect in silico prediction of the membrane permeability of drugs.

Keywords: PAMPA | Digital simulation | non-Bornian model

1. Introduction

The study of absorption, distribution, metabolism, and excretion (ADME) of drugs is a key element in the development of pharmaceuticals. The initial process of ADME, i.e., absorption, is primarily important and has been extensively studied by means of several techniques described below.

In classical quantitative structure–activity relationship (QSAR) studies,[1–3] the partition coefficient of compounds between 1-octanol and water ($\log P_{\mathrm{oct}}$) was found to show a linear free-energy relationship (LFER) with the biological activities. Since then, $\log P_{\mathrm{oct}}$ has been extensively utilized as the scale of hydrophobicity or membrane permeability of compounds.[4]

More recently, parallel artificial membrane permeation assay (PAMPA) using an artificial lipid membrane was developed for evaluating the membrane permeability of drugs.[5–10] Analogous methods were also developed by using human colon adenocarcinoma (Caco-2) cells,[11,12] Madin–Darby canine kidney (MDCK) cells,[13,14] etc. However, among these methods, PAMPA has most frequently been used for a high throughput screening method, since it is less costly, less labor intensive, and more reproducible.

On the other side, much attention should be paid to the polarized oil|water (O|W) interface, which has been used as a simple biomembrane model.[15] Since 1992, several research groups[16–27] have used ion-transfer voltammetry (ITV) to study the transfer of ionic drugs across O|W interfaces. The standard ion-transfer potential ($\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$) determined by ITV has been claimed to be a good measure for the hydrophobicity or biomembrane permeability of ionic drugs and thus for their pharmacological activities. In our recent study,[27] the transfer of nine amine drugs at the 1,2-dichloroethane (DCE)|W interface was observed by ITV to determine $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$ for the protonated amines and also the distribution coefficient (K_{D}) of their neutral forms. It was then reported that the PAMPA permeability coefficient (P_{pampa}) showed a clear and characteristic dependence on $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$ or $\log K_{\mathrm{D}}$.

Furthermore, digital simulation was applied by Velický et al.[28] and recently by our group[29] for studying the permeation dynamics of drugs in PAMPA. In our paper, it was demonstrated that the characteristic dependence of P_{pampa} on the hydrophobicity of drug molecules can be simulated by using the distribution coefficient ($K_{\mathrm{D,M}}$) of a drug at the lipid membrane (M)|W interface. It was also shown that the near sigmoidal pH-dependence of $\log P_{\mathrm{pampa}}$[30–32] can be explained in terms of the "pH partition hypothesis" proposed by Brodie and coworkers.[33,34] According to this hypothesis, a drug molecule as a weak electrolyte exists in aqueous solution as neutral and ionized species in equilibrium, and then only the neutral species is able to get through the lipid membrane. This theoretical analysis suggested that the $K_{\mathrm{D,M}}$ value can be determined from the experimental $\log P_{\mathrm{pampa}}$ vs. pH curve with the help of the digital simulation technique.

In this study, we have performed PAMPA measurements to examine the pH dependence of $\log P_{\mathrm{pampa}}$ for nine amine drugs (their chemical structures are shown in Supporting Information, Figure S1). Then, the $K_{\mathrm{D,M}}$ values for the amine drugs could be obtained with the help of the previously developed digital simulation technique.[29] As described above, the values of $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$ and K_{D} at the DCE|W interface were previously determined (or estimated) by ITV for the amine drugs.[27] Furthermore, we have optimized the measurement conditions as mentioned elsewhere,[35] and thus obtained more reliable data of $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi°$ and K_{D} will be reported in this paper. On the other hand, the values of $\log P_{\mathrm{oct}}$ for the amine drugs were reported by Fujikawa et al.[8] In this study, we have examined the correlations among the distribution/transport parameters, i.e., P_{pampa}, $K_{\mathrm{D,M}}$, $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$, K_{D}, and $\log P_{\mathrm{oct}}$. The results have clearly suggested that the parameters in the DCE|W system ($\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ and K_{D}) show relatively high correlation with $K_{\mathrm{D,M}}$ than $\log P_{\mathrm{oct}}$ in the 1-octanol|W system. Additionally, we would like to stress that $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ can be estimated rather accurately by using the non-Bornian solvation model, which was recently proposed by our group.[35,36] Taking this into account, we have found a possibility of perfect in silico evaluation of the membrane permeability of drugs.

2. Experimental Section

Chemicals. Amine drugs, (**1**) desipramine, (**2**) imipramine, (**3**) labetalol, (**4**) propranolol, (**5**) alprenolol, (**6**) acebutolol, (**7**) nadolol, (**8**) verapamil, and (**9**) diltiazem, were purchased from Sigma–Aldrich or Santa Cruz Biotechnology as hydrochloride salts (or the neutral form for **7**) and were used as received. Dimethyl sulfoxide (DMSO; Kishida Chemical), dodecane (Tokyo Chemical Industry), L-*α*-phosphatidylcholine (Sigma–Aldrich), and other reagents were of analytical grade and used as received.

PAMPA Measurements. A 96-well microtiter plate (Millipore, MATRNPS50) and a 96-well microtiter filterplate (Millipore, MAIPNTR10) were assembled into a "sandwich" such that each composite well was separated by a hydrophobic polyvinylidene fluoride (PVDF) filter plate (0.45 μm pores). Referring to a previous paper,[5] the filter plate was impregnated with 5 μL of a 10% (w/v) dodecane solution of L-*α*-phosphatidylcholine. Into donor (D) and acceptor (A) compartments placed on the bottom and top of the filter plate, respectively, 300 μL of a phosphate-buffered saline (PBS) solution (containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.47 mM KH_2PO_4) was poured. The pH of D- and A-compartments was adjusted to values between 2 and 13 by using hydrochloric acid or sodium hydroxide. A test compound dissolved in DMSO in advance was added to the bottom (D-) compartment at 500 μM so that the DMSO concentration became 5% (v/v). The permeability assay was started at the time t = 0 by placing the 96-well microtiter filterplate (with A-compartments) on the microtiter plate (with D-compartments). After leaving the sandwiched assembly at room temperature (25 ± 2 °C) for t = 2, 4, and 24 h, the concentration of the test compound in the A-compartment, $c_{\mathrm{A}}(t)$, was determined by taking an aliquot (200 μL) of the solution, adding 250 μL of DMSO[37] and 50 μL of 0.2 M phosphate buffer, and then measuring the absorbance at 280–300 nm using Hitachi U-3000 spectrophotometer with a 10 mm-path quartz micro cell (GL Sciences, M20-UV-1). Calibration curves were prepared at each pH value. Thus, in order to achieve accurate and reliable measurements, we did not employ any automatic measurement instrument.

Determination of P_{pampa}. The experimental values of P_{pampa} were obtained from the time courses of $c_{\mathrm{A}}(t)$ under various pH conditions by fitting the respective plots using a conventional equation:

$$c_{\mathrm{A}}(t) = \frac{M}{V_{\mathrm{D}}+V_{\mathrm{A}}}\left[1 - \exp\left\{-P_{\mathrm{pampa}}A\left(\frac{1}{V_{\mathrm{D}}}+\frac{1}{V_{\mathrm{A}}}\right)t\right\}\right] \qquad (1)$$

This equation was previously derived by Palm et al.,[38] who assumed a "steady-state" diffusion profile across the membrane. Rigorously, the "steady-state" assumption is not valid in the lag time before the steady state is reached. As described previously,[29] however, the effect of the relatively short lag time is not very significant in the determination of P_{pampa}. In eq 1, M is the "total" amount (mol) of the drug in the system (vide infra); V_{D} and V_{A} are the volumes (cm^3) of D- and A-compartments; A is the area (cm^2) of the membrane. Considering the experimental conditions used, we set A = 0.4 cm^2 and $V_{\mathrm{D}} = V_{\mathrm{A}}$ = 0.3 cm^3 and then performed a regression analysis for each $c_{\mathrm{A}}(t)$ vs. t plot by using the software IGOR Pro 6.3 (WaveMetrics).

Avdeef et al.[39] described that M is not the total amount of the drug, but should be decreased by the amount of drug retention in the membrane. The amount of retention is different for different drugs and essentially dependent on t. In this study, however, M was used as a fitting parameter independent of t, for evaluating P_{pampa} in a conventional manner. As an example, the $c_{\mathrm{A}}(t)$ vs. t plots obtained for desipramine (**1**) at different pH's (2.5–10.3) are shown with their fitting curves in Supporting Information, Figure S2. Thus, the values of P_{pampa} at different pH's could be determined for all the amine drugs tested.

3. Digital Simulation

Model. For digital simulation, we used the model proposed previously.[29] In the model, a drug added to D-compartment is distributed to the membrane and then transported by diffusion via the membrane to A-compartment. Herein, we set an unstirred water layer (UWL) on each side of the membrane, which has a constant thickness δ; it is empirically known that a diffusion layer does not increase beyond a certain thickness owing to natural convection in the bulk solution. Thus the drug is transported by diffusion in the respective UWL's as well as in the membrane. The diffusion process is then simulated by the finite-difference method, which has been widely used for the study of electrode reactions.[40–42] Here, each UWL has been divided into five[43] volume elements with a constant thickness, $\Delta x = \delta/5$. The membrane has likewise been divided into five elements with a constant thickness, $\Delta x_{\mathrm{M}} = \delta_{\mathrm{M}}/5$ (where δ_{M} is the membrane thickness).

Parameters. Table 1 shows the parameters commonly used in the digital simulation for all the drugs studied. The time interval Δt was chosen as 10 s so that the model diffusion coefficients, $\mathbf{D}_{\mathrm{m}}$ ($= D_{\mathrm{iff}}\Delta t/(\Delta x)^2$) in UWL and $\mathbf{D}_{\mathrm{m}}^{\mathrm{M}}$ ($= D_{\mathrm{iff}}^{\mathrm{M}}\Delta t/(\Delta x_{\mathrm{M}})^2$) in M, do not exceed 0.5 for stable calculation (vide infra).[41] The thickness of UWL on each side of the membrane, δ, cannot be clearly determined. The δ value in PAMPA experiments without stirring was estimated to be 2000–4000 μm,[44] and also that in an unstirred Caco-2 assay to be 1544 μm.[45] On the other hand, much smaller values (153 μm or less) were reported for planar bilayer lipid membranes, being dependent on the solute diffusion coefficient.[46] Referring to these values and learning through trial and error, we have found an optimal value for δ (=550 μm), which achieved stable calculation and then gave realistic simulation results shown below. The thickness of the membrane, d_{M}, was simply estimated from the radius of the membrane (0.36 cm) and the volume of the membrane solution (5 μL).

The diffusion coefficient of a drug in either D- or A-compartment, D_{iff} (in $\mathrm{cm}^2\ \mathrm{s}^{-1}$), was evaluated from the molecular weight (MW) by using:

$$\log D_{\mathrm{iff}} = -4.113 - 0.4609 \log \mathrm{MW} \qquad (2)$$

This equation was empirically derived by Avdeef et al.[44] using the data for 96 molecules with MW's less than 5000. Rigorously, D_{iff} should be dependent on pH, since there is a

Table 1. Parameters Commonly Used in the Digital Simulation for All the Drugs Studied

Parameter	Symbol	Value	Unit
Initial concentration	$c^t_{\mathrm{D,b}}(t = 0\ \mathrm{s})$	5.0×10^{-7}	mol cm^{-3}
Time interval	Δt	10	s
Thickness of D- or A-compartment		1	cm
Thickness of UWL	δ	0.055	cm
Number of division of UWL		5	
Thickness of volume element in UWL	Δx	0.011	cm
Thickness of M	d_M	0.012	cm
Number of division of M		5	
Thickness of volume element in M	Δx_M	0.0024	cm

certain difference between the diffusion coefficients for neutral and protonated forms of a basic drug. However, in the present simulation, the D_iff values obtained from eq 2 were regarded as the averaged or effective diffusion coefficient.

The diffusion coefficient of a drug in the membrane, $D^\mathrm{M}_\mathrm{iff}$, is considered to be much smaller than D_iff (in water), because the PVDF filter membrane used has a reticular or net-like configuration. In this simulation, we assumed $D^\mathrm{M}_\mathrm{iff} = D_\mathrm{iff}/1000$ as one of the best choice by trial and error.

As an example, the D_iff value for **1** (with MW = 266) is obtained from eq 2 as 5.88×10^{-6} cm^2 s^{-1}, which gives $D^\mathrm{M}_\mathrm{iff} = 5.88 \times 10^{-9}$ cm^2 s^{-1}. These diffusion coefficients lead to the corresponding model diffusion coefficients, $\mathbf{D}_\mathrm{m} = 0.49$ and $\mathbf{D}^\mathrm{M}_\mathrm{m} = 0.01$, which do not exceed 0.5 so as to achieve stable calculation.

Simulation Method. The details of the simulation method and the required mathematical equations are as reported previously.[29] Simulation calculations were made by using a spreadsheet application (Microsoft Excel for Mac 2011).

4. Results and Discussion

Determination of $\log K_\mathrm{D,M}$. As a typical example, the values of $\log P_\mathrm{pampa}$ for **1** are plotted against pH (Figure 1). As reported by previous authors,[30–32] a sigmoid-like dependence was observed. In our previous study,[29] we could predict such a sigmoid-like dependence by means of digital simulation based on the “pH partition hypothesis” by Brodie and his coworkers.[33,34] According to the hypothesis, a basic drug (D) exists in aqueous solution as neutral and protonated forms in equilibrium ($\mathrm{D} + \mathrm{H}^+ \rightleftharpoons \mathrm{DH}^+$); DH^+ is unable to get through a lipid membrane, whereas D, being more lipophilic, is able to be absorbed by digestive tract.

On the assumption that only D can get through the membrane, we have defined an apparent or effective distribution coefficient:

$$K'_\mathrm{D,M} = f_\mathrm{n} K_\mathrm{D,M} \tag{3}$$

where f_n is the fraction of the neutral species, being given for a basic drug by

$$f_\mathrm{n} = \frac{[\mathrm{D}]}{[\mathrm{D}]+[\mathrm{DH}^+]} = \frac{1}{1+10^{\mathrm{p}K_\mathrm{a}-\mathrm{pH}}} \tag{4}$$

Here, pK_a is the acid dissociation index of the conjugate acid (DH$^+$) of the basic drug. Using eq 3 with eq 4, we can simulate the pH dependence of $\log P_\mathrm{pampa}$ by taking into account the acid–base equilibrium.

Figure 1 also shows simulation curves obtained for three different values of $\log K_\mathrm{D,M}$ (=4.9, 5.9, and 6.9). As seen in the figure, the experimental data could be most satisfactorily reproduced for $\log K_\mathrm{D,M} = 5.9$. With increasing or decreasing $\log K_\mathrm{D,M}$ by one unit, the calculated $\log P_\mathrm{pampa}$ vs. pH curve is shifted to lower or higher pH’s by one unit. It should here be noted that the experimental $\log P_\mathrm{pampa}$ started to increase at around pH 5, where the fraction of the neutral form of **1** (with pK_a = 10.65 [8]) is extremely small: $f_\mathrm{n} = 2.2 \times 10^{-6}$. This clearly shows that the membrane permeability is considerably enhanced by the higher hydrophobicity of the drug (having $\log K_\mathrm{D,M} = 5.9$). In the same manner as in Figure 1, the $\log K_\mathrm{D,M}$ values for all the amine drugs could be determined by fitting the experimental $\log P_\mathrm{pampa}$ vs. pH plot to the theoretical curve obtained by digital simulation. In Table 2, the $\log K_\mathrm{D,M}$ values thus determined are shown with other parameters including MW, pK_a, $\Delta^\mathrm{W}_\mathrm{O}\phi°$, $\log K_\mathrm{D}$, and $\log P_\mathrm{oct}$.

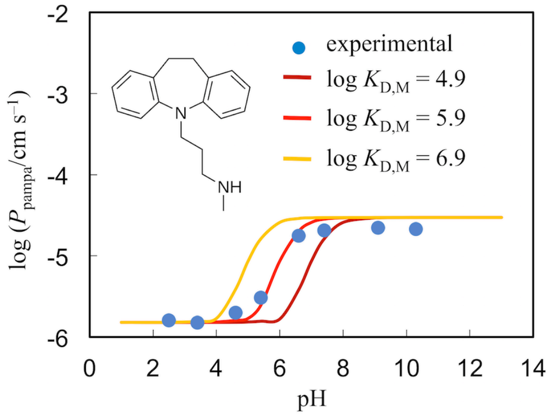

Figure 1. pH dependence of $\log P_\mathrm{pampa}$ for **1**. Solid circles: experimental data; solid lines: theoretical curves obtained by digital simulation with $\log K_\mathrm{D,M}$ = 4.9, 5.9, and 6.9.

We would like to add that in the sigmoid-like pH dependence of $\log P_\mathrm{pampa}$ (for basic drugs), the upper limit at higher pHs should be dependent on various parameters including the diffusion coefficient and hydrophobicity of drugs and the thickness of the membrane and/or UWL.[29]

Dependence of $\log P_\mathrm{pampa}$ on $\log K'_\mathrm{D,M}$. In the previous paper,[29] we discussed the dependence of $\log P_\mathrm{pampa}$ on the hydrophobicity of amine drugs by using their “effective” distribution coefficient, $K'_\mathrm{D} = f_\mathrm{n} K_\mathrm{D}$, at the DCE|W interface. A clear and characteristic dependence of $\log P_\mathrm{pampa}$ on $\log K'_\mathrm{D}$ could be shown by using the literature values[8] of $\log P_\mathrm{pampa}$ at pH 7.3. In this study, we have investigated the dependence of $\log P_\mathrm{pampa}$ on $\log K'_\mathrm{D,M}$, on the basis of our PAMPA measurements and the digital simulation analysis. In Fig. 2, the values of $\log P_\mathrm{pampa}$ at pH 7.4 for the amine drugs are plotted against $\log K'_\mathrm{D,M}$ at the same pH. The solid line represents the theoretical curve, which has been obtained by using the

Table 2. Parameters for the Amine Drugs Studied

	Drug	MW [a]	$\mathrm{p}K_\mathrm{a}$ [a]	$\Delta_\mathrm{O}^\mathrm{W}\phi°(\mathrm{exp})$ /V [b]	$\Delta_\mathrm{O}^\mathrm{W}\phi°(\mathrm{theor})$ /V [c]	$\log K_\mathrm{D}$ [b]	$\log K_\mathrm{D,M}$ [d]	$\log P_\mathrm{oct}$ [a]	$\log P_\mathrm{pampa}$ (pH 7.4)[d]
1	Desipramine	266	10.65	–0.003	0.022	3.5	5.9	4.54	–4.68
2	Imipramine	280	9.40	–0.069	–0.074	3.6	5.1	4.44	–4.63
3	Labetalol	328	9.40	0.186	0.222	1.0	3.5	3.09	–4.87
4	Propranolol	259	9.60	0.085	0.116	2.3	5.0	2.98	–4.74
5	Alprenolol	249	9.60	0.100	0.105	1.2	3.6	2.89	–4.78
6	Acebutolol	336	9.41	0.117	0.109	1.0	3.1	1.71	–5.09
7	Nadolol	309	9.67	0.283	0.279	–0.6 [e]	2.7	0.71	–5.76
8	Verapamil	455	8.66	–0.076	–0.127	4.0	6.0	3.79	–4.67
9	Diltiazem	415	8.06	–0.099	–0.102	4.2	5.3	2.80	–4.65

a) From Fujikawa et al.[8] b) Newly determined by ITV in a similar manner as reported previously.[27,35] c) Theoretical values obtained by using the non-Bornian solvation model.[35] d) Determined in this study. e) Estimated by using eq 7.

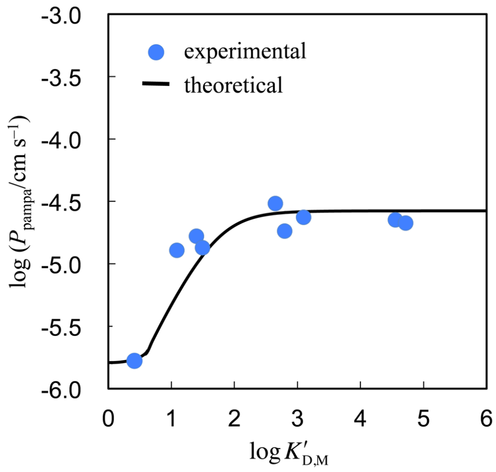

Figure 2. Plots of the experimental values of $\log P_\mathrm{pampa}$ against $\log K'_\mathrm{D,M} = \log(f_\mathrm{n}K_\mathrm{D,M})$ for the nine amine drugs at pH 7.4. The solid line represents the theoretical curve obtained by digital simulation.

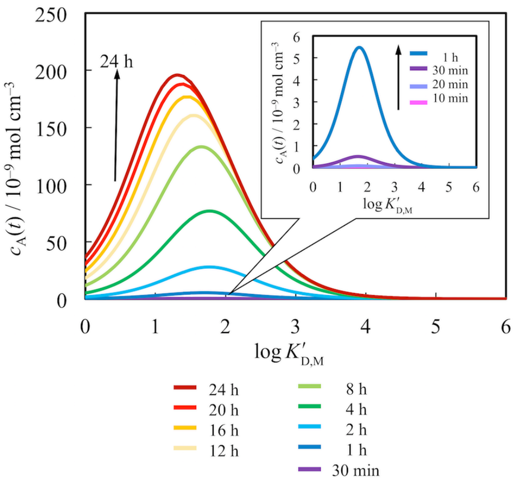

Figure 3. Simulated dependence of $c_\mathrm{A}(t)$ on $\log K'_\mathrm{D,M}$ for different incubation times (t = 10 min to 24 h).

averaged value of D_iff for the nine amine drugs (5.36×10^{-6} cm^2 s^{-1}).[47] As expected, the experimental plots were well fitted to the theoretical curve obtained by digital simulation.[48]

As clearly shown in Figure 2, in the lower range of $\log K'_\mathrm{D,M}$ (i.e., for less hydrophobic drugs), $\log P_\mathrm{pampa}$ is increased with the increase in $\log K'_\mathrm{D,M}$, showing a higher permeability of the drug. However, when $\log K'_\mathrm{D,M}$ exceeds a certain threshold (here, ca. 2), $\log P_\mathrm{pampa}$ shows signs of "leveling off". This shows that the drugs having the hydrophobicity higher than a certain level may show the optimal membrane permeability. Nevertheless, as also claimed in the previous paper,[29] the amount of a drug transported through the membrane is reduced by increasing the hydrophobicity of the drug molecule. Figure 3 shows the dependence of $c_\mathrm{A}(t)$ on $\log K'_\mathrm{D,M}$ for different incubation times (t = 10 min to 24 h). As seen in the figure, the $c_\mathrm{A}(t)$ vs. $\log K'_\mathrm{D,M}$ curve has a maximum at around $\log K'_\mathrm{D,M} = 1.5$, showing that the drug amount transported is considerably lowered even after long incubation time (e.g., t = 24 h). This is ascribed to the retention of drug molecules in the membrane, as discussed in detail previously.[29] Thus, considerably hydrophobic drugs with $\log K'_\mathrm{D,M} > 2$ give the highest value of $\log P_\mathrm{pampa}$ (Figure 2), however they cannot be easily transported through the membrane, from a quantitative viewpoint (Figure 3). Judging from the results shown in Figures 2 and 3, a drug with $\log K'_\mathrm{D,M} \approx 1.5$ would achieve the best permeability, not only from a kinetic but also from a quantitative viewpoint.

Correlation Studies. Correlations of the $\log K_\mathrm{D,M}$ values determined by PAMPA measurements have been studied with other hydrophobicity parameters that include $\Delta_\mathrm{O}^\mathrm{W}\phi°$, K_D, and $\log P_\mathrm{oct}$.

First, we should stress that $\log K_\mathrm{D,M}$ showed a LFER with $\Delta_\mathrm{O}^\mathrm{W}\phi°$ and $\log K_\mathrm{D}$, which were determined by ITV with the DCE|W interface:

$$\log K_\mathrm{D,M} = -0.833\Delta_\mathrm{O}^\mathrm{W}\phi° + 4.95 \quad (n = 9;\ R^2 = 0.754) \quad (5)$$

$$\log K_\mathrm{D,M} = 0.701\log K_\mathrm{D} + 2.89 \quad (n = 9;\ R^2 = 0.891) \quad (6)$$

These and the following correlations are also shown by Figures S3–S9 in Supporting Information.

Thus, decent or relatively high coefficients of determination (R^2) were found between $\log K_\mathrm{D,M}$ and the hydrophobicity parameters of ionic and neutral forms of the amine drugs, i.e., $\Delta_\mathrm{O}^\mathrm{W}\phi°$ and $\log K_\mathrm{D}$. This suggests that the solvation state of the drugs in the lipid-containing dodecane solution (in PAMPA) should be similar to that in the DCE solution (in ITV).

We would like to add that a good linear correlation was found between $\log K_\mathrm{D}$ and $\Delta_\mathrm{O}^\mathrm{W}\phi°$ for all the amine drugs except for nadolol (**7**):

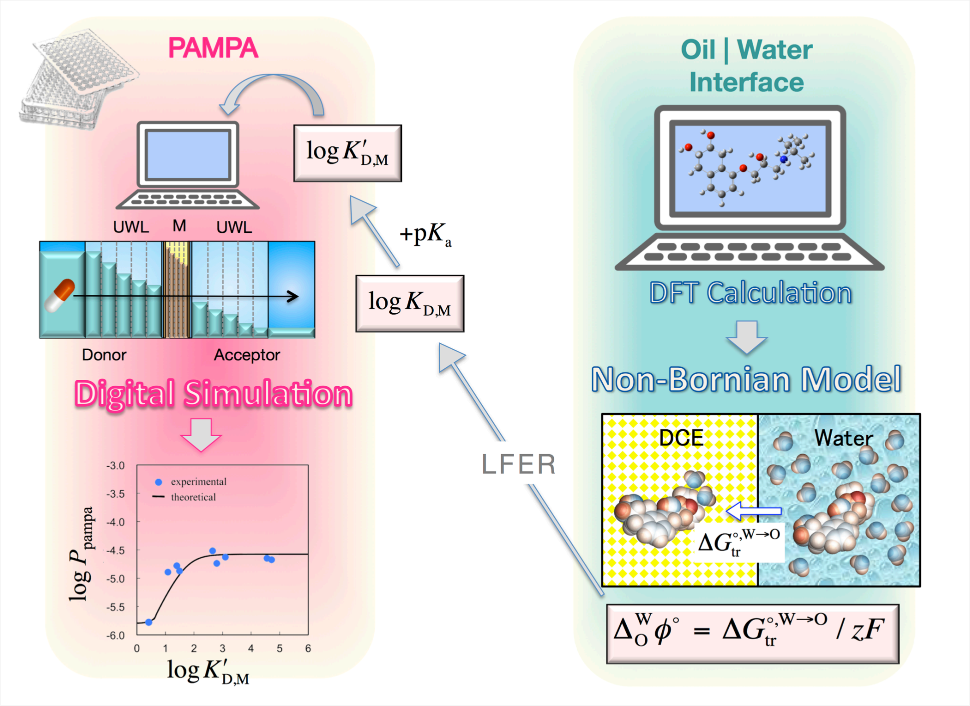

Figure 4. In silico prediction of the membrane permeability of drugs.

$$\log K_{\mathrm{D}} = -12.56\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ} + 2.98 \quad (n = 8;\ R^2 = 0.918) \qquad (7)$$

The $\log K_{\mathrm{D}}$ for **7**, which could not be determined experimentally, was estimated as –0.6 by eq 7.

On the other hand, $\log K_{\mathrm{D,M}}$ showed a lower correlation with $\log P_{\mathrm{oct}}$ conventionally used as the hydrophobicity scale of compounds in QSAR studies.[4]

$$\log K_{\mathrm{D,M}} = 0.828\log P_{\mathrm{oct}} + 1.99 \quad (n = 9;\ R^2 = 0.663) \qquad (8)$$

Similarly, $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ and $\log K_{\mathrm{D}}$ showed worse correlations with $\log P_{\mathrm{oct}}$:

$$\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ} = -0.0778\log P_{\mathrm{oct}} + 0.291 \ (n = 9;\ R^2 = 0.538) \qquad (9)$$

$$\log K_{\mathrm{D}} = 1.08\log P_{\mathrm{oct}} - 0.990 \quad (n = 9;\ R^2 = 0.621) \qquad (10)$$

As described in the previous paper,[27] these results can be elucidated by different solvation states of the amine drugs in 1-octanol; that is, the amine drugs should be solvated differently by forming one to three hydrogen bond(s) or not. Such solvation behavior in 1-octanol seems to differ from that in DCE, or possibly in the lipid-containing dodecane membrane. A similar view was first presented by Kontturi and Murtomäki.[16]

In Silico Prediction. As shown above, the developed digital simulation method enables us to evaluate the membrane permeability of a drug in PAMPA, provided that the $K_{\mathrm{D,M}}$ value is known for the drug. It is not necessarily feasible to obtain the $K_{\mathrm{D,M}}$ value using another measurement, however we can use the linear correlation between $\log K_{\mathrm{D,M}}$ and $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ (eq 5) to estimate the former from the latter.

As described previously,[35,36] $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ is related to the standard Gibbs energy of ion transfer at the O|W interface as $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ} = \Delta G_{\mathrm{tr}}^{\circ,\mathrm{W}\to\mathrm{O}}/zF$ (z, the charge number including the sign; F, Faraday constant), and can be predicted theoretically by using a recently proposed solvation model called the non-Bornian model. In the model, local electric fields on the surface of an ion are first evaluated by density functional theory (DFT) calculation. Using the calculation result and an experimental data set of $\Delta G_{\mathrm{tr}}^{\circ,\mathrm{W}\to\mathrm{O}}$, a multivariate regression analysis is performed to get the semi-empirical equation for $\Delta G_{\mathrm{tr}}^{\circ,\mathrm{W}\to\mathrm{O}}$. This equation is available for rather accurate prediction of $\Delta G_{\mathrm{tr}}^{\circ,\mathrm{W}\to\mathrm{O}}$ for organic ions. In the DCE|W system,[35] the mean absolute error of $\Delta G_{\mathrm{tr}}^{\circ,\mathrm{W}\to\mathrm{O}}$ was 1.9 and 3.1 kJ mol^{-1} for 26 organic cations (including **1**–**9**) and 24 anions, respectively. These errors correspond to the errors of ~20 and ~30 mV in $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$, being comparable with the typical error in ITV measurements (10 mV or so). In Table 2, the theoretical values of $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ for the amine drugs are shown as well as the experimental values. The theoretical values were obtained by using the regression equation and coefficients reported previously (i.e., Eq. (12) and the coefficients in Table 3 in the previous paper[35]). An excellent linear correlation was found between the theoretical and experimental values:

$$\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}(\mathrm{theor}) = 1.079\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}(\mathrm{exp}) - 0.0017$$

$$(n = 9;\ R^2 = 0.971) \qquad (11)$$

As described above, the non-Bornian model enables us to predict very accurately the value of $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^{\circ}$ for the ionic form of a drug. Then, eq 5 is available for evaluation of $K_{\mathrm{D,M}}$, which can be corrected for the acid–base equilibrium to give $K'_{\mathrm{D,M}}$. Using this value, we can predict the permeation dynamics of the drug in PAMPA measurements, by using the developed digital simulation technique. Such in silico prediction of membrane permeability of drugs (illustrated in Figure 4) would be useful for labor-saving, shortening the development period, and reducing the cost in drug discovery. For achieving this goal at a higher level, it would be desirable to refine the correlation equation (eq 5 with $n = 9$; $R^2 = 0.754$) by performing both ITV and PAMPA measurements for much more drugs (including anionic drugs as well). The study is currently in progress.

5. Conclusion

Using the previously developed digital simulation method, the value of $\log K_{\mathrm{D,M}}$ for a drug can be determined from the experimental pH dependence of $\log P_{\mathrm{pampa}}$. The $\log K_{\mathrm{D,M}}$ values thus determined for nine amine drugs have shown a decent correlation ($R^2 = 0.754$) with their experimental $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^\circ$ values. On the other side, using the non-Bornian solvation model, the $\Delta_{\mathrm{O}}^{\mathrm{W}}\phi^\circ$ values can be theoretically predicted with sufficient confidence (eq 11). These findings suggest a possibility of in silico prediction not only of the values of $\log P_{\mathrm{pampa}}$ but also of the detailed permeation dynamics of drugs in PAMPA experiments.

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Supporting Information
Figures S1–S9 are available as Supporting Information. This material is available on http://dx.doi.org/??????.

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47) In our previous digital simulation analysis,[29] it was suggested that the variation of D_{iff} for the amine drugs only slightly affects the $\log P_{\mathrm{pampa}}$ vs. $\log K_{\mathrm{D}}'$ curve.
48) The theoretical curve was drawn by using the theoretical values of $\log P_{\mathrm{pampa}}$, which were obtained from regression analyses with eq 1 for the time courses of $c_{\mathrm{A}}(t)$ obtained by digital simulation.[29] For these regression analyses the lag time before the steady state is reached was neglected, because its effect on $\log P_{\mathrm{pampa}}$ would not be so significant.

Graphical Abstract

A Strategy for in Silico Prediction of the Membrane Permeability of Drugs

Yu Fujii, Erina Yoshida, and Toshiyuki Osakai

Perfect in silico prediction of the membrane permeability of drugs may be achieved by combining the non-Bornian solvation model for evaluating the standard ion-transfer potential at the oil|water interface and the digital simulation technique for calculating the membrane permeability in the parallel artificial membrane permeation assay (PAMPA).