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Evaluation of the artificial membrane permeability of drugs by digital simulation

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ABSTRACT

A digital simulation method has been developed for evaluating the membrane permeability of drugs in the parallel artificial membrane permeation assay (PAMPA). The simulation results have shown that the permeability coefficient (log P_{pampa}) of drugs is linearly increased with increasing their distribution coefficient (log $K_{D,M}$) to the lipid membrane, i.e., the hydrophobicity of the drug molecules. However, log P_{pampa} shows signs of leveling off for highly hydrophobic drugs. Such a dependence of log P_{pampa} is in harmony with the reported experimental data, and has been well explained in terms of the change in the rate-determining step from the diffusion in the membrane to that in the unstirred water layer (UWL) on both sides of the membrane. Additionally, the effects of several factors, including lag time, diffusion coefficient, pH, and pK_a, on the permeability coefficient have been well simulated. It has thus been suggested that the proposed method should be promising for in silico evaluation of the membrane permeability of drugs.

1. Introduction

Prediction of the absorption, distribution, metabolism, and excretion (ADME) of drugs is crucial in the initial step of drug development. For this purpose, quantitative structure–activity relationship (QSAR) studies have been carried out extensively. In the classical QSAR study, Hansch and coworkers (Hansch et al., 1962; Hansch and Fujita, 1964; Fujita et al., 1964) claimed that there was a linear free energy relationship (LFER) between the biological activity and the partition coefficient (log P_{oct}) between 1-octanol and water for compounds. Since then, log P_{oct} has been extensively used, in QSAR studies, as the scale of hydrophobicity or membrane permeability of compounds (Leo et al., 1971). More recently, the parallel artificial membrane permeation assay (PAMPA) using an artificial lipid membrane was developed for evaluating the membrane permeability of drugs in a more realistic manner (Kansy et al., 1998; Kerns, 2001; Sugano et al., 2001; Fujikawa et al., 2005, 2007; Avdeef et al., 2007). Similar methods were developed by using human colon adenocarcinoma (Caco-2) cells (Hilgers et al., 1990; Artursson, 1990) and Madin–Darby canine kidney (MDCK) cells (Cho et al., 1989; Irvine et al., 1999). Among these methods, however, PAMPA has been most frequently used for a high throughput screening, because it is less costly, less labor intensive, and more reproducible.

On the other hand, much attention has been paid to the polarized oil|water (O|W) interface (or the interface between two immiscible electrolyte solutions; ITIES) as a simple biomembrane model (Volkov, 2001). Several research groups, including Helsinki groups (Kontturi and Murtomäki, 1992; Mälkiä et al., 2004), Arai et al. (1993, 1994), Ding and Osakai (2001), Ding et al. (2001), and Lausanne groups (Reymond et al., 1999; Gobry et al., 2001; Bouchard et al., 2001, 2002; Ulmeanu et al., 2003), employed an electrochemical technique called "ion-transfer voltammetry (ITV)" to study the transfer of ionic drugs at O|W interfaces. It has been found that the standard ion-transfer potential ($\Delta_O^W\phi^\circ$) determined by ITV is a good measure for the hydrophobicity or biomembrane permeability of ionic drugs and thus for their pharmacological activities.

In our recent study (Nakamura and Osakai, in press), the transfer of amine drugs at the 1,2-dichloroethane (DCE)|W interface was studied to determine the $\Delta_O^W\phi^\circ$ of protonated amines and the distribution coefficient (K_D) of their neutral forms. It was then found that the PAMPA permeability coefficient (P_{pampa}) showed a clear and characteristic dependence on $\Delta_O^W\phi^\circ$ or log K_D. With increasing $\Delta_O^W\phi^\circ$ negatively or increasing log K_D positively (i.e., with increasing the hydrophobicity of drug molecules), log P_{pampa} is linearly increased, but shows signs of leveling off for highly hydrophobic drugs. Similar dependence was observed for the Caco-2 cell permeability on log P_{oct} (pH 7.4) (Krämer, 1999; Avdeef et al., 2005). It has been pointed out that for highly hydrophobic drugs, their diffusion process in the unstirred water layer (UWL) or the aqueous boundary layer (ABL) on both sides of the membrane is

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rate limiting (Karlsson and Artursson, 1991; Adson et al., 1995; Bermejo et al., 2004; Avdeef et al., 2004, 2005).

In this study, in order to understand the characteristic dependence of log P_{pampa} on the hydrophobicity of drugs (i.e., $\Delta_O^W\phi^*$ or log K_D), we have developed a digital simulation method for studying the permeation dynamics of drugs in PAMPA. Our method was successfully used to reproduce the experimental dependence of log P_{pampa} on the hydrophobicity of drug molecules. Furthermore, the time-dependent drug distribution in the PAMPA system could be well simulated with physicochemical parameters. So far, the permeation process of drugs across lipid membranes has been discussed generally by using a steady-state assumption. However, this assumption is, of course, not valid before the steady state is established. Velicky et al. (2010) employed a commercial software to simulate the time-dependent permeation profiles, though under limited conditions that the transport at the membrane|-acceptor solution interface is blocked. Then these authors made an interesting discussion about the lag time that is required to establish the steady state.

2. Digital simulation

2.1. Permeation model

PAMPA is usually performed in a 96-well microtiter plate format, in which the donor (D), filter, and acceptor (A) parts are constructed. In the filter part, a hydrophobic membrane is prepared by adding phospholipids dissolved in organic solvent, and then the permeability of drugs via the membrane is evaluated. Specifically, the amount of a drug transported from D- to A-compartment is monitored by means of UV absorption spectroscopy. For the present simulation, we used the model shown in Fig. 1. In this model, we set an UWL (or ABL) in each side of the membrane. A drug molecule is transported by diffusion in the respective UWL's as well as in the membrane. In this study, the diffusion process has been simulated by the finite-difference method (Feldberg, 1969; Bard and Faulkner, 1980; Britz, 2005), which has been widely used for the study of electrode reactions. It is here assumed that each UWL has a constant thickness (i.e., $\delta = 0.1$ cm). This assumption should be valid not only for stirred conditions but also for unstirred conditions; it is empirically known that a diffusion layer does not increase beyond a certain thickness owing to natural convection in bulk solution.

2.2. Simulation method

The diffusion of a drug in D-compartment has been simulated as described below. The diffusion processes in A-compartment and in the membrane can likewise be simulated (the details are shown in Supplementary material).

The drug added to D-compartment is firstly distributed to the membrane, and then transported via the membrane to A-compartment, so that a concentration profile is set up across the membrane as shown in Fig. 1. In the present simulation model, each UWL has been divided into five[1] volume elements with a constant thickness, $\Delta x = \delta/5 = 0.02$ cm. The membrane has likewise been divided into five elements with a constant thickness, $\Delta x_M = d_M/5 = 0.002$ cm (here, d_M is the membrane thickness). The concentration profile across the membrane has been approximated by a series of discrete changes in concentration.

According to Fick's first law, the flux (J) of a diffusing species is given by

$$J = -D_{iff}\left(\frac{dc}{dx}\right) \tag{1}$$

where D_{iff} and (dc/dx) are, respectively, the diffusion coefficient and concentration gradient of the diffusing specie. In the finite-difference method, the flux from the k-th volume element to the $(k+1)$-th volume element is expressed by

$$J_{D,k}^t = -D_{iff}\frac{\Delta c_{D,k}^t}{\Delta x} = -\frac{D_{iff}}{\Delta x}\left(c_{D,k+1}^t - c_{D,k}^t\right) \quad (k = 1,2,3,4) \tag{2}$$

where $c_{D,j}^t$ ($j = k$ or $k+1$) is the averaged concentration in the j-th volume element of the D-side UWL, at an arbitrary time (t) measured from the start of the permeation experiment.

Considering that Fick's second law:

$$\frac{\partial c}{\partial t} = D_{iff}\frac{\partial^2 c}{\partial x^2} \tag{3}$$

is derived from the equation:

$$\frac{\partial c}{\partial t}dx = -D_{iff}\left[\left(\frac{\partial c}{\partial x}\right)_x - \left(\frac{\partial c}{\partial x}\right)_{x+dx}\right] \tag{4}$$

we can express the concentration change ($\Delta c_{D,k}^{t+\Delta t}$) in the k-th volume element in a small time interval (Δt) as

$$\frac{\Delta c_{D,k}^{t+\Delta t}}{\Delta t} = \frac{J_{D,k-1}^t - J_{D,k}^t}{\Delta x} \quad (k = 2,3,4) \tag{5}$$

Then, from Eqs. (2) and (5), the drug concentration in the k-th volume element at the time $t + \Delta t$ is given by using the concentrations in the $(k-1)$-th, k-th, and $(k+1)$-th volume elements at the time t:

$$c_{D,k}^{t+\Delta t} = c_{D,k}^t + \Delta c_{D,k}^{t+\Delta t} = c_{D,k}^t + \frac{D_{iff}\Delta t}{(\Delta x)^2}\left(c_{D,k+1}^t - 2c_{D,k}^t + c_{D,k-1}^t\right) \quad (k = 2,3,4) \tag{6}$$

In the model shown in Fig. 1, we have set additional two layers having no volume on each side of the D-side UWL|membrane (M) interface. The drug concentration in the volume element with $k = 1$ at $t + \Delta t$ is then expressed as

$$c_{D,1}^{t+\Delta t} = c_{D,1}^t + \Delta c_{D,1}^{t+\Delta t} = c_{D,1}^t + \frac{D_{iff}\Delta t}{\Delta x^2}\left(c_{D,2}^t - c_{D,1}^t\right) + \frac{J_{D,int}^t \Delta t}{\Delta x} \tag{7}$$

where $J_{D,int}^t$ is the flux from the interface to the D-solution, being given by

$$J_{D,int}^t = -\frac{2D_{iff}}{\Delta x}\left(c_{D,1}^t - c_{D,int}^t\right) \tag{8}$$

Here, $c_{D,int}^t$ is the drug concentration in the D-side layer on the UWL|M interface. Note also that the flux in the opposite direction, i.e., to the interface, is given by $-J_{D,int}^t$. In a similar manner to Eq. (8), the flux from the membrane to the interface is given by

$$J_{M,int(D)}^t = -\frac{2D_{iff}^M}{\Delta x_M}\left(c_{M,int(D)}^t - c_{M,1}^t\right) \tag{9}$$

where $c_{M,1}^t$ and $c_{M,int(D)}^t$ denote the drug concentrations in the volume element with $k = 1$ and in the interfacial layer of the membrane, respectively; and D_{iff}^M is the diffusion coefficient in the membrane.

Provided that the drug is not adsorbed at the W|M interface, the continuity of the flux is established:

$$J_{D,int}^t = J_{M,int(D)}^t \tag{10}$$

[1] Considering the speed of calculation in the computer used, we set the number of division to five. A larger number of division would be desirable for more rigorous calculation.