ADMET

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Reversed phase parallel artificial membrane permeation assay for log *P* measurement

Zihao Song, Katsuhide Terada, Kiyohiko Sugano*

Department of pharmaceutics, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba, 274-8510, Japan

*Corresponding Author: E-mail: kiyohiko.sugano@phar.toho-u.ac.jp; Tel.: +81 47 472 1494

Abstract

A reversed phase parallel artificial membrane permeation assay (RP-PAMPA) was newly invented for log P measurement. An oil/water/oil sandwich was constructed using a conventional PAMPA instrument. 1 % agarose was used to improve the physical stability of the water phase. A linear correlation between log P and the apparent permeability was observed in the -0.24 < log P < 2.85 region (R^2 = 0.98). RP-PAMPA was also applied to pKa measurement.

Keywords

octanol, partition, parallel artificial membrane permeation assay, pK_a, drug

Introduction

High throughput physicochemical profiling of a drug is still challenging in early drug discovery. Various methods have been proposed for octanol – water partition coefficient (log *P*), solubility, and pK_a measurements [1]. The parallel artificial membrane permeation assay (PAMPA) has been widely used in drug discovery as PAMPA is compatible with high throughput screening (HTS) [2,3]. In normal phase (NP-) PAMPA methods, a lipid phase is immobilized on a filter (usually a 96 well filter plate) and the permeability of a drug across the lipid membrane is measured. Previously, Faller et al. applied NP-PAMPA to log *P* measurement [4]. The apparent permeability (P_{app}) across the octanol impregnated filter membrane was found to correlate with logP. However, the P_{app} – log *P* curve showed a bell-shaped relationship with a plateau around log *P* = 1. Therefore, it was impossible to estimate the log *P* values around log *P* = 1. A bell-shaped relationship between P_{app} and lipophilicity of drugs is usually observed in NP-PAMPA [5].

The purpose of the present study was to overcome the drawback of NP-PAMPA for log *P* measurement. A reversed phase PAMPA (RP-PAMPA) method for log *P* measurement was newly invented. In RP-PAMPA, an oil/water/oil sandwich was constructed using a conventional PAMPA instrument (Figure 1). In addition, RP-PAMPA was applied for pK_a measurement, especially for low solubility compounds.
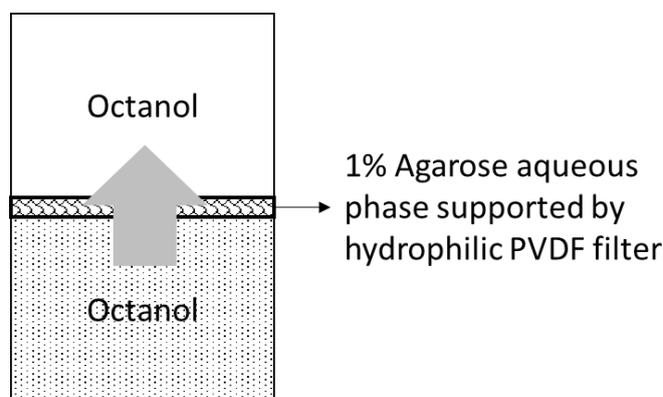
Figure 1. Schematic configuration of RP-PAMPA

Materials and Methods

Materials

Octanol, pentoxifylline, 1-naphthol, dipyridamole, and acid blue 9 were purchased from Tokyo chemical industry (Tokyo, Japan). Agar powder, agarose S, agarose H, prednisone, sulfamethoxazole, carbamazepine, caffeine, chlormphenicol, ethanol, trisodium citrate, sodium dihydrogenphosphate, disodium hydrogenphosphate, sodium hydroxide solution, propranolol hydrochloride, warfarin sodium, piroxicam, and ketoprofen were purchased from Wako pure chemicals (Tokyo, Japan). Phenacetin was purchased from Yamamoto Corporation (Osaka, Japan). The other reagents were of analytical grade.

P_{app} measurement

The schematic configuration of RP-PAMPA is shown in Figure 1. In RP-PAMPA, the water phase (water membrane) was immobilized on the hydrophilic filter with the aid of agarose. Agarose S was dissolved in hot water or a buffer at 1.0 % and then poured into a hydrophilic filter (Multi Screen-HV, pore size 0.48 μm, low protein binding, Millipore). A model drug was dissolved in octanol at 10 mM and added to the donor plate (downside, 300 μL). The filter plate was then put on the donor plate. The filter plate was filled with 200 μL of octanol. After 16 hour incubation at room temperature (25 ± 1 °C), both octanol phases were diluted tenfold by ethanol and the drug concentrations in the donor and accepter sides were measured by UV spectroscopy. For pK_a measurement sodium - phosphate and sodium - citric acid buffers (100 mM of anion species) were used to construct the water membrane. P_{app} was calculated as previously reported [6]:

$$P_{app} = \frac{-\ln(1 - C_A / C_{equilibrium})}{A \times (1/V_D + 1/V_A) \times t} \tag{1}$$

$$C_{equilibrium} = \left(C_D \times V_D + C_A \times V_A\right) / \left(V_D + V_A\right) \tag{2}$$

where C_A and C_D are the drug concentrations in the donor and acceptor phases at time t, respectively. V_D and V_A are the volumes of the donor and acceptor phases, respectively. A is the membrane surface area (0.28 cm^2).

Results

Construction of RP-PAMPA

We first investigated the stability of the water phase (water membrane) constructed on the hydrophilic

filter with the aid of agarose. 1.0 % concentration was selected to enable pipetting of the hot sol phase while maintaining the physical strength of the agarose gel. It was found that at least 30 μL of 1 % agarose was required to provide sufficient physical strength for P_{app} measurement. The ager powder was found to form a less stable water phase compared to Agarose S and H. In a preliminary study, it was found that more than 10 hours were required to achieve a steady – state flux across the water phase (data not shown). Therefore, the Agarose S 30 μL water membrane and 16 hour incubation time were employed in the following studies.

Log P_{app} – log P relationship

The log P_{app} and log P data are summarized in Table 1 [1,7,8]. Figure 2 shows the correlation between log P_{app} and log P. A linear correlation was observed in the -0.24 < log P < 2.85 region (R^2 = 0.98). The slope of the log-log plot was -0.48.

Table 1. P_{app} and log P

Drug	log P (literature)[a]	log P_{app} (cm s^{-1}, mean ± S.D., N = 6)
phenacetin	1.58	-6.38 ± 0.04
caffeine	0.10	-5.53 ± 0.02
carbamazepine	2.1	-6.52 ± 0.04
prednisone	1.56	-6.25 ± 0.01
chloramphenicol	1.14	-6.13 ± 0.01
sulfamethoxazole	0.70	-5.96 ± 0.02
1-naphthol	2.85	-6.95 ± 0.03
pentoxifylline	0.38	-5.78 ± 0.02

[a] Refs. [1,7,8]
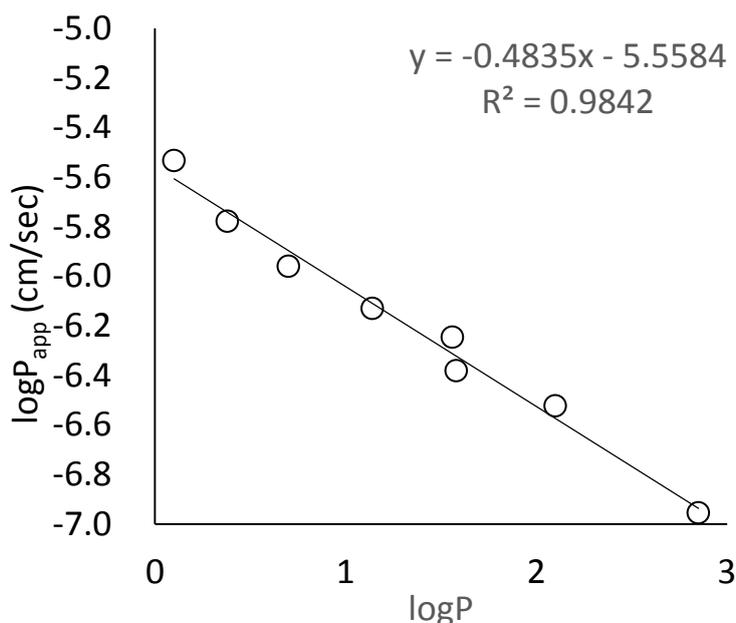
Figure 2. Log P – log P_{app} relationship.

pH - P_{app} profile

The pH - P_{app} profiles were shown in Figure 3 and Table 2. The pK_a values were obtained as the intersection of the slope and the horizontal lines. Estimated and literature pK_a values are shown in Table 3 [1,9].
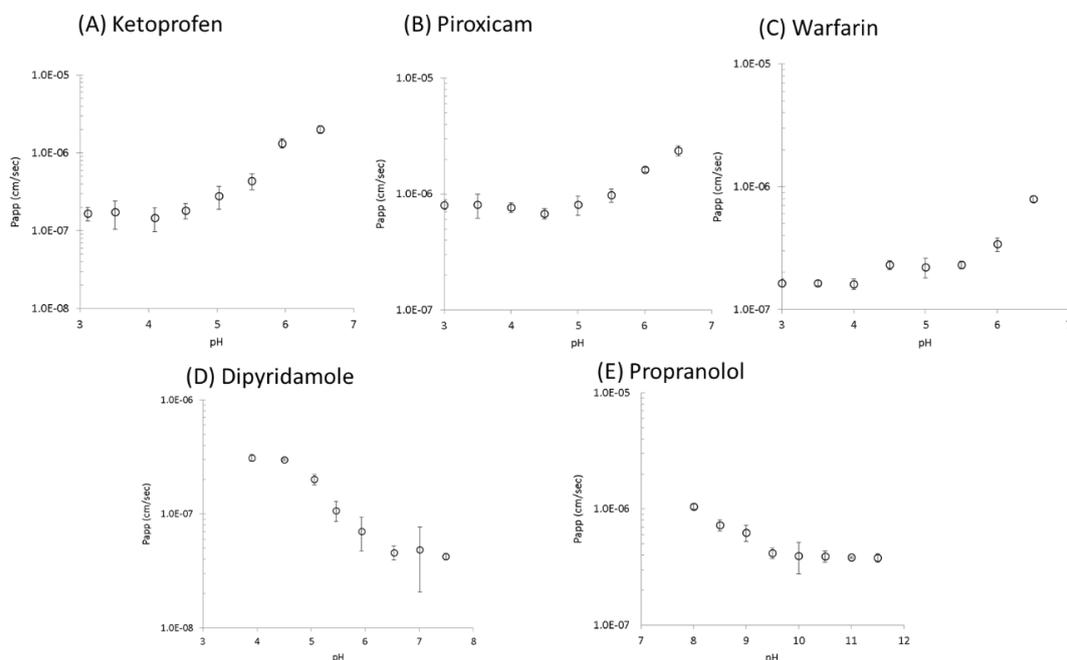
Figure 3. pH – P_{app} relationship. Mean ± S.D. N = 3.

Table 2. P_{app} of dissociable drugs at each pH[a]

Compound	pH	P_{app} (10^{-6} cm sec^{-1})	Compound	pH	P_{app} (10^{-6} cm sec^{-1})
Ketoprofen	3.1	0.17 ± 0.03	Dipyridamole	3.9	0.31 ± 0.02
(log P = 3.2)	3.5	0.17 ± 0.07	(log P = 3.9)	4.5	0.30 ± 0.01
	4.1	0.15 ± 0.05		5.1	0.20 ± 0.02
	4.5	0.18 ± 0.04		5.5	0.11 ± 0.02
	5.0	0.28 ± 0.09		5.9	0.07 ± 0.02
	5.5	0.44 ± 0.10		6.5	0.05 ± 0.01
	6.0	1.33 ± 0.18		7.0	0.05 ± 0.03
	6.5	2.00 ± 0.23		7.5	0.04 ± 0.00
Piroxicam	3.0	0.81 ± 0.07	Propranolol	8.0	1.05 ± 0.06
(log P = 2.0)	3.5	0.81 ± 0.19	(log P = 2.9)	8.5	0.72 ± 0.08
	4.0	0.77 ± 0.07		9.0	0.63 ± 0.10
	4.5	0.68 ± 0.07		9.5	0.42 ± 0.04
	5.0	0.81 ± 0.15		10.0	0.40 ± 0.12
	5.5	0.98 ± 0.13		10.5	0.39 ± 0.04
	6.0	1.62 ± 0.08		11.0	0.38 ± 0.01
	6.5	2.37 ± 0.24		11.5	0.38 ± 0.03
Warfarin	3.0	0.16 ± 0.01			
(log P = 3.1)	3.5	0.16 ± 0.01			
	4.0	0.16 ± 0.02			
	4.5	0.23 ± 0.02			
	5.0	0.22 ± 0.04			
	5.5	0.23 ± 0.02			
	6.0	0.34 ± 0.04			
	6.5	0.80 ± 0.05			

[a] Mean ± S.D. N = 3. Measured at 25 °C. The buffer concentration was 100 mM.

Table 3. pK_a values

Drug	This study[a]	Literature[b]
Ketoprofen	4.9	4.0
Piroxicam	4.8	4.7
Warfarin	5.7	5.0
Dipyridamole	5.9	6.1
Propranolol	9.4	9.5

[a] Measured at 25 °C. The buffer concentration was 100 mM.
[b] Refs. [1], [9].

Discussion

In this study, RP-PAMPA for log *P* measurement was investigated for the first time. 1.0 % agarose was used to improve the physical stability of the water membrane. The mesh size of agarose is significantly larger than the size of drug molecules so that it does not affect the diffusion coefficient of drugs [10]. By using RP-PAMPA, log *P* in the -0.24 < log *P* < 2.85 range can be accurately measured. The measurable range can be expanded by using a more sensitive quantitation method such as LC-MS. The slope of the P_{app} – log *P* relationship was 0.45, which is significantly smaller than 1. If P_{app} follows the solubility – partition theory for membrane permeation, i.e., $P_{app} = PD/h$ where *D* is the diffusion coefficient and *h* is the thickness of the membrane, the slope of the log-log plot should be unity [11]. The reason for this deviation is not clear.

Previously, Kwon et al. reported a poly(dimethylsiloxane)(PDMS) permeation assay, which might be regarded as a kind of reversed phase membrane permeation assay [12]. However, the configuration of the PDMS permeation assay was largely different from the one used in the present study that is usually referred as PAMPA. In the PDMS permeation assay, a side-by-side single diffusion chamber was employed. A PDMS membrane was put between two chambers filled with aqueous bulk fluids. In addition, PDMS disks were added to both the donor and acceptor sides as dosing and sampling (extracting) phases, respectively. The aqueous phases were stirred by magnetic stirrers. In the PDMS permeation assay, a good correlation was observed between log P_{app} and log *P* in the range of log *P* > 3 even though PDMS was used instead of octanol as the oil phase.

For low solubility drugs, it has been difficult to measure pK_a by using conventional methods such as pH titration. The pH – solubility profile can be used to estimate pK_a for low solubility drugs [13,14]. However, this method may not be accurate due to aggregate formation, low detection limit, etc. In RP-PAMPA, a drug is solubilized in the organic solvent phase. Therefore, it would become possible to measure pK_a for low solubility drugs by using RP-PAMPA. As the pH of the water membrane was changed in RP-PAMPA, the pH – P_{app} relationship should become a mirror image of that for NP-PAMPA. However, the pH – P_{app} relationship deviated from the Henderson – Hasselbalch equation. Therefore, the pK_a values of the model drugs were estimated as the intersection of the slope and the horizontal lines. The pK_a values of acidic drugs were underestimated by the RP-PAMPA method. The reason for this deviation is not clear. One possible reason may be that the incubation time of 16 hours might not be sufficient to achieve a steady state at pH > pK_a for acids. The pK_a of diclofenac has been reported to be ca. 4.0 in most cases in the literature. However, pK_a of 5.7 was obtained from the pH-solubility profile [15]. The P_{app} value at a pH where a drug molecule is undissociable (intrinsic water permeability, $P_{w,int}$) also correlated with log *P*. However, $P_{w,int}$ deviated from the log P_{app} – log *P* line for the undissociable drugs about 0.3 log unit. The difference of the water membrane (pure water vs. a buffer) could be a reason for the discrepancy. The present RP-PAMPA method

needs to be improved for pK_a measurement in the future.

In conclusion, in the present study, RP-PAMPA for log *P* measurement was constructed for the first time. 1.0 % agarose can be used to stabilize the water membrane. RP-PAMPA was applied to log *P* and pK_a measurements. As PAMPA is compatible with the current HTS instrument, RP-PAMPA will be a useful tool in drug discovery.

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