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Original scientific paper

Effect of albumin on artificial membrane permeation of acidic drugs

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Abstract

Background and purpose: The free fraction theory (FFT) plays a central role in pharmacokinetics and pharmacodynamics. However, for acidic drugs, deviations from FFT have been reported in cell-based assays involving albumin binding. The purpose of the present study was to investigate the effect of albumin on the passive membrane permeation of acidic drugs in the parallel artificial membrane permeation assay (PAMPA). **Experimental approach:** Eight acidic drugs (atorvastatin, bosentan, entacapone, fluvastatin, meloxicam, pitavastatin, rosuvastatin, and tolbutamide) were used as model drugs. The PAMPA membrane consisted of phospholipids dissolved in decane. The effective permeation coefficient (P_e) was measured in the absence and presence of 4 % bovine serum albumin (BSA) in the donor solution. The unbound fraction (f_u) was determined using equilibrium dialysis. Concentration dependence and phospholipid-composition dependence of P_e were examined. **Key results:** P_e was substantially reduced by the addition of BSA, showing good consistency with FFT. The minor deviation from FFT was explained by a concentration-dependent increase in the membrane permeability of unbound drug molecules, except for rosuvastatin. The effect of BSA on the permeability of rosuvastatin was influenced by phospholipid composition, possibly suggesting albumin-phospholipid interactions. **Conclusion:** PAMPA permeation showed good consistency with FFT. The slight deviations from FFT observed for the low-permeability drugs can be explained by the concentration dependence of unbound drug permeability, except for rosuvastatin.

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Keywords

Free fraction theory; bovine serum albumin; phospholipids; lipophilicity

Introduction

The free fraction theory (FFT) plays a central role in pharmacokinetics and pharmacodynamics [1-3]. In plasma and cytosol, drug molecules exist in an equilibrium between protein-bound and unbound states. According to FFT, only unbound (free) drug molecules can permeate cellular membranes and interact with pharmacological targets and metabolic enzymes [1]. FFT has proven valid in most cases [1]; however, several exceptions have also been documented [4-7]. In hepatic drug clearance, deviations from FFT have been reported in both hepatic uptake [8-12] and metabolism [5,6,13]. This phenomenon is referred to as albumin-mediated uptake [14,15]. Albumin-mediated uptake has been experimentally observed mainly for acidic drugs that are substrates of organic anion transporting polypeptides (OATPs) [16]. However, albumin-mediated uptake has also been observed in wild-type human embryonic kidney 293 cells (wt-HEK293), which do not express OATPs [8]. Various molecular-level mechanisms have been proposed to explain the deviation from FFT [6,10,16]. Because unknown transporter(s) may be involved in wt-HEK293, it is important to

evaluate the impact of albumin on drug membrane permeation using a membrane system that isolates passive transport [17,18]. The parallel artificial membrane permeation assay (PAMPA) has been widely used in drug discovery and development [19-21]. A PAMPA membrane is suitable for evaluating passive transport exclusively. Previously, the effect of albumin in the acceptor solution has been investigated using a PAMPA system without phospholipids [22,23]. However, it has remained unclear whether PAMPA permeation is affected by albumin in the donor solution when using a PAMPA system containing phospholipids.

The purpose of the present study was to investigate the effect of albumin on the PAMPA permeation of acidic drugs regarding FFT. In this study, eight acidic drugs (atorvastatin, bosentan, entacapone, fluvastatin, meloxicam, pitavastatin, rosuvastatin, and tolbutamide) were used as model drugs (Figure 1, Table 1). The effect of albumin on the uptake in wt-HEK293 cells has been reported for these model drugs except meloxicam [8]. Phospholipid-decane solutions were used to construct the PAMPA membrane [24]. Bovine serum albumin (4 %) was added to the donor solution.

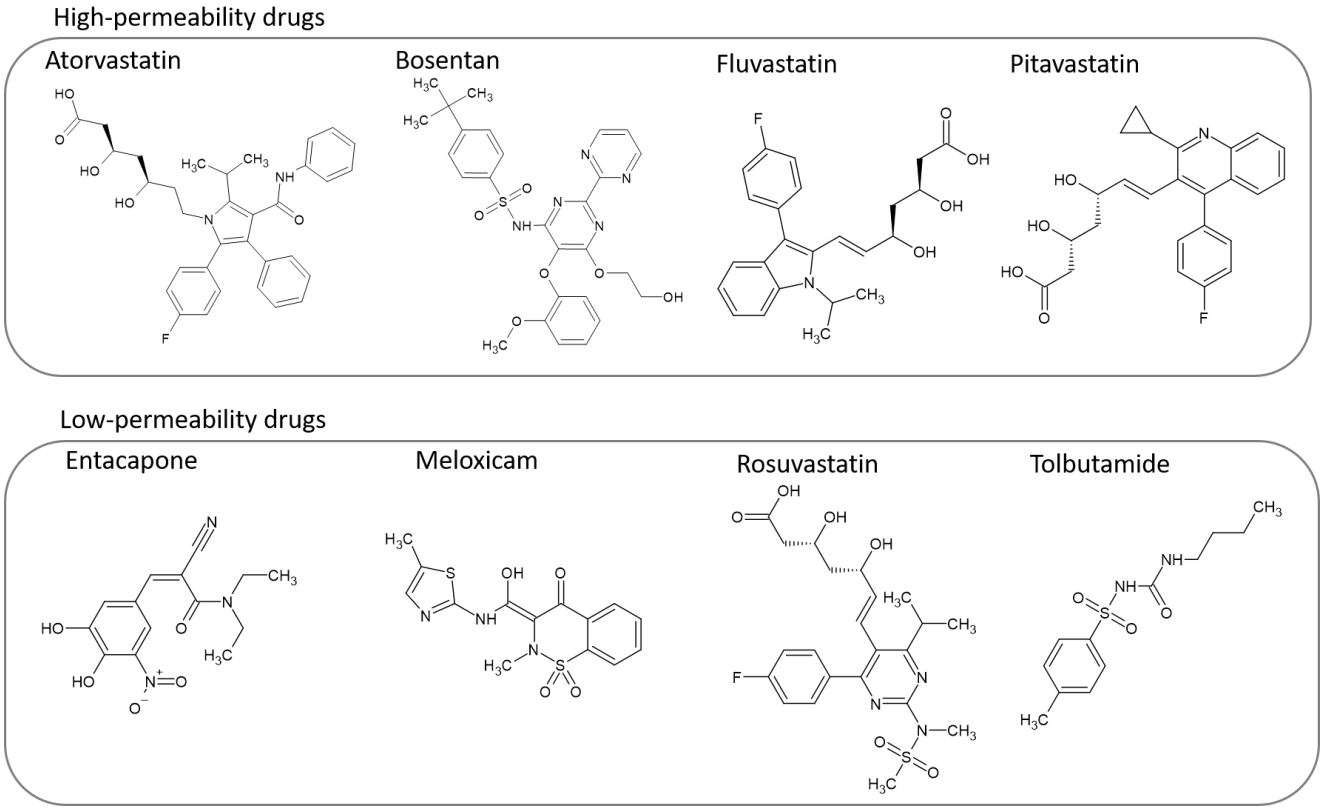


Figure 1. Chemical structures of model drugs

Table 1. Physicochemical properties of model drugs

Drug	MW	pK_a	log P_{oct}	Log $D_{oct,pH\ 7.4}$[b]	ref. (pK_a)	ref. (log P_{oct})
High-permeability drugs						
Atorvastatin	559	4.2 (acid)[a]	4.0	0.80	[25]	[25]
Bosentan	552	4.9 (acid) (37 °C, *I* = 0.168 M)	3.8	1.27	[26]	[27]
Fluvastatin	411	4.3 (acid) (25 °C, *I* = 0.15 M)	4.2	1.11	[28]	[28]
Pitavastatin	421	4.4 (acid), 5.4 (base)[a]	3.0 [c]	1.03	[29]	[29]
Low-permeability drugs						
Entacapone	305	4.5 (acid)[a]	2.2	-0.24[d]	[30]	[31]
Meloxicam	351	4.2 (acid) (23 °C, *I* = 0.1 M)[a]	2.7	0.07[d]	[32]	[32]
Rosuvastatin	482	4.3 (acid) (25 °C, *I* = 0.15 M)	2.5[e]	-0.3[d]	[28]	[33]
Tolbutamide	270	5.2 (acid) (24 °C, *I* = 0.01 M)	2.2	-0.01	[28]	[34]

[a]Temperature and ionic strength were not reported; [b]Octanol-water distribution coefficient (log D_{oct}) at pH 7.4 calculated from log P_{oct} and pK_a (unless otherwise noted): [c]Estimated from log D_{oct} = 1.49 at pH 6.93, assuming negligible distribution of the zwitterionic species in octanol: [d]Experimental value; [e]Log P_{oct} was estimated from log D_{oct} and pK_a.

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Experimental

Material

Bovine serum albumin (BSA), tolbutamide, decane, acetonitrile, and 0.1 vol.% trifluoroacetic acid - acetonitrile was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Atorvastatin calcium trihydrate, fluvastatin sodium, meloxicam, rosuvastatin calcium, and pitavastatin calcium were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Bosentan hydrate was purchased from Combi-Blocks Inc (CA, USA). Entacapone was extracted from the Entacapone tablet purchased from KYOWA Pharmaceutical Industry Co., Ltd (Tokyo, Japan). Phosphate-buffered saline (PBS, 0.14 M sodium chloride, 0.0027 M HCl, 0.010 M Phosphate) was purchased from TOHO Co., Ltd (Tokyo, Japan). 0.1 vol% trifluoroacetic acid-distilled water was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). A cellulose dialysis membrane (MWCO 3500) was purchased from As-One Corporation (Osaka, Japan). Soybean lecithin (SLP-white and SLP-PC 70) (Table 2) was provided by Tsuji Oil Mills Co., Ltd. (Mie, Japan). Egg yolk lecithin (PC-98N) was purchased from Kewpie Corporation (Tokyo, Japan).

Table 2. Composition of phospholipid[a]

Phospholipid	Content, %		
	SLP-white	SLP-PC70	PC-98N
Phosphatidylcholine	24 to 32	65 to 75	98.8
Phosphatidylethanolamine	20 to 28	10 to 15	-
Phosphatidylinositol	12 - 20	0 to 1	-
Phosphatidic acid	8 to 15	1 to 3	-
Lysophosphatidylcholines	1 to 5	1 to 5	0.9

[a]Taken from the product information provided by the manufacturer

Methods

Measurement of albumin unbound fraction by equilibrium dialysis

Albumin unbound fraction (f_u) was measured by equilibrium dialysis using a side-by-side chamber with the acceptor chamber sandwiched between two donor chambers (SANPLATEC Co., Ltd., Osaka, Japan). The area of the dialysis membranes was 4.0 cm^2 (2.0 cm^2 per membrane). The fluid volume was 0.9 mL in the acceptor and 3.0 mL in the donor chambers (1.5 mL per chamber). Each drug solution (0.1 mM in pH 7.4 PBS) with 4 % BSA (0.60 mM) was added to the donor side. PBS without a drug was added to the acceptor chamber. After incubation for 24 h at 37 °C, the drug concentration in the acceptor chamber was measured by HPLC (Shimadzu Prominence LC-20 series, column: ZORBAX Eclipse Plus (C18 2.1×50 mm, 3.5 µm) (Agilent Technologies), flow rate: 0.6 mL min^{-1}, column temperature: 40 °C, and injection volume of 10 µL). The mobile phase composition and the detection wavelength are listed in Supplemental Material Table S1.

Parallel artificial membrane permeation assay

The PAMPA sandwich consisted of a 96-well filter plate (hydrophobic PVDF, 0.45 µm) and a PAMPA acceptor plate (Merck Millipore, MA, USA). The acceptor plate was filled with 300 µL of PBS. The filter of the donor well was coated with 5 µL (10 % SLP-white) or 10 µL (5 % SLP-white, 5 % SLP-PC70 or 5 % PC-98N) of a phospholipid-decane solution. A drug solution (0.1 mM (except for the concentration-dependency study), PBS, 200 µL) with or without 4 % BSA was added to the donor compartment. The pH was set to 7.4 on both donor and acceptor sides. The PAMPA sandwich was then incubated at 37 °C for 4 h (atorvastatin, bosentan, fluvastatin, and pitavastatin) or 20 h (entacapone, meloxicam, rosuvastatin, and tolbutamide). The donor solution (0.1 mL) was mixed with acetonitrile (0.1 mL) and centrifuged at 2000 g for 10 min (ForceMini, BM Equipment Co., Ltd., Tokyo, Japan). The supernatant was filtered through a 0.22 µm PVDF membrane (Millipore Corporation, Billerica, MA, USA). The first few drops of the filtrate were discarded to minimize

adsorption to the filter. Drug concentrations were measured by HPLC. The HPLC conditions were the same as those described above. The effective permeability (P_e) was calculated using the following equations [28].

$$P_e = -\frac{2.303V_D}{At}\left(\frac{1}{1+r_v}\right)\log\left(-r_v+\left(\frac{1+r_v}{1-R_t}\right)\frac{C_D(t)}{C_D(0)}\right) \quad (1)$$

$$R_t = 1-\frac{C_D(t)}{C_D(0)}-\frac{1}{r_v}\frac{C_A(t)}{C_D(0)} \quad (2)$$

$$r_v = \frac{V_D}{V_A} \quad (3)$$

where A is the filter surface area (0.266 cm^2), V_D and V_A are the fluid volumes, mL of the donor and acceptor phases, respectively, t is the incubation time, $C_D(t)$ and $C_A(t)$ are the concentrations of a drug in the donor and acceptor phases, respectively, R_t is the membrane retention factor and r_v is the volume ratio. It should be noted that $C_D(t)$ is the sum of BSA-bound and unbound drug molecules in the donor solution, and P_e is defined by this drug concentration. The details for the derivation of these equations have been reported elsewhere [17]. In PAMPA, P_e is usually calculated from one-time point data. In typical PAMPA experiments, the steady state is achieved within 0 to 45 min. Therefore, P_e was calculated from one-time-point data at > 2 h.

Results and discussion

Effect of BSA on PAMPA permeability

The effective permeability of the model drugs is summarized in Table 3. In the absence of BSA, atorvastatin, bosentan, fluvastatin, and pitavastatin showed high permeability (P_e > 10×10^{-6} cm s^{-1}), whereas entacapone, meloxicam, rosuvastatin, and tolbutamide showed low permeability (P_e < 5×10^{-6} cm s^{-1}). In the presence of BSA, P_e was substantially reduced in all cases (Figure 2). R_{PAMPA} is defined as the ratio of the PAMPA permeability values measured with and without BSA. The ratio R_{PAMPA}/f_u quantifies the extent of deviation from FFT. For comparison, the same ratio for the wt-HEK293 cells ($R_{wt\text{-}HEK293}/f_u$) was calculated using the data reported in the literature [8]. R_{PAMPA}/f_u was close to unity (0.9 to 1.6) and substantially smaller than $R_{wt\text{-}HEK293}/f_u$, indicating that the deviation from FFT in PAMPA is minimal relative to that in wt-HEK293 cells (Figure 3).

Table 3. Effective permeability and unbound fraction (f_u) (Mean ± S.D., N = 3)

Drug	P_e (×10^{-6} cm s^{-1})[a]		R_{PAMPA}	f_u [b,c]	R_{PAMPA} / f_u[c]	$R_{wt\text{-}HEK293}$ / f_u[d]
	BSA-	BSA+				
High-permeability drugs						
Atorvastatin	19.8 ± 0.4	1.19 ± 0.03	0.060 ± 0.001	0.054 ± 0.001	1.1	2.4
Bosentan	26.4 ± 2.2	1.30 ± 0.03	0.049 ± 0.001	0.055 ± 0.001	0.9	0.8
Fluvastatin	39.9 ± 0.7	1.11 ± 0.03	0.028 ± 0.001	0.029 ± 0.000	1.0	0.9
Pitavastatin	24.9 ± 0.5	1.13 ± 0.07	0.045 ± 0.003	0.037 ± 0.000	1.2	3.3
Low-permeability drugs						
Entacapone	1.19 ± 0.03	0.09 ± 0.00	0.074 ± 0.004	0.047 ± 0.000	1.6	6.7
Meloxicam	2.37 ± 0.06	0.06 ± 0.00	0.024 ± 0.001	0.021 ± 0.000	1.2	-
Rosuvastatin	0.60 ± 0.01	0.14 ± 0.01	0.24 ± 0.01	0.17 ± 0.00	1.4	1.4
Tolbutamide	3.12 ± 0.05	0.60 ± 0.02	0.19 ± 0.01	0.18 ± 0.00	1.1	5.5

[a]PAMPA membrane: 10 % SLP-white/decane, 5 µL. Drug concentration: 0.1 mM; [b]Measured by equilibrium dialysis; [c]With bovine serum albumin; [d]With human serum albumin [8]

In the 96-well PAMPA assay, the effective permeability of high-permeability drugs (P_e > 10×10^{-6} cm s^{-1}) is influenced by the unstirred water layer (UWL) [35]. This is also evident from the fact that P_e reaches a plateau as lipophilicity increases in this study (Figure 4). In such cases, the slow diffusion coefficient of BSA-bound drug molecules in the UWL affects P_e, and the slow dissociation rate of the drug from BSA may also affect P_e [36].

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The theoretical analysis of this reaction-diffusion system in the UWL is of great interest; however, it lies outside the scope of the present study.

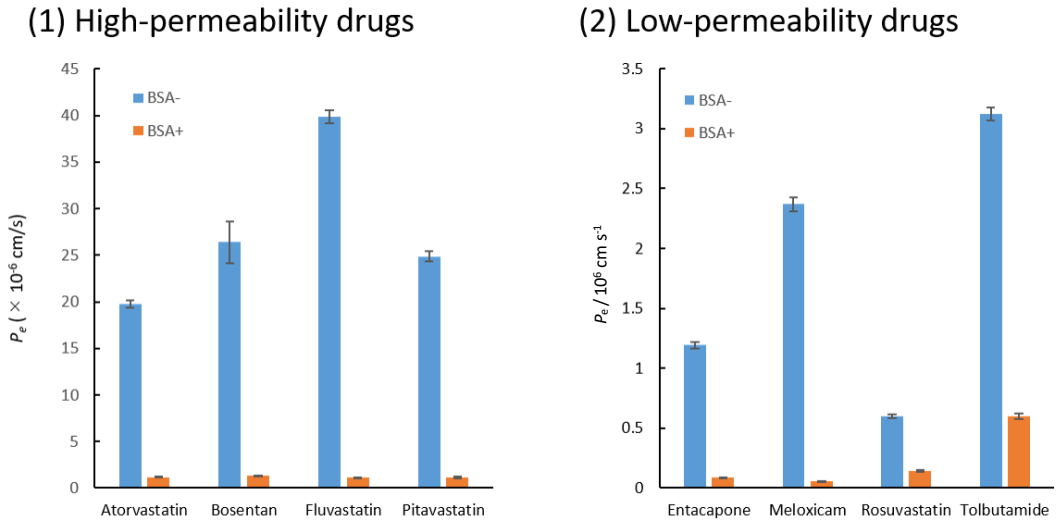


Figure 2. Effect of BSA on PAMPA permeability. 4 % BSA was added to the donor side of the PAMPA membrane. Mean ± S.D., N = 3

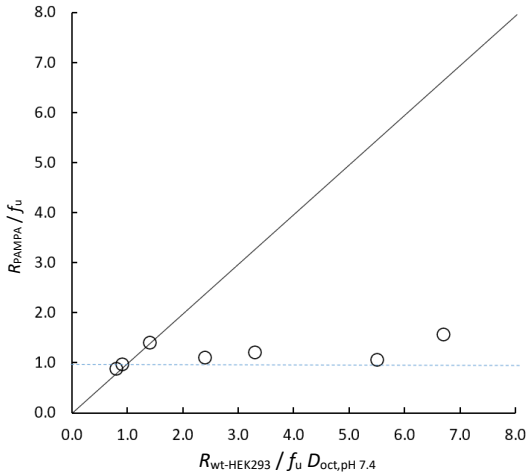


Figure 3. Correlation between $R_{wt\text{-}HEK293}/f_u$ and R_{PAMPA}/f_u. The black solid line indicates $R_{wt\text{-}HEK293}/f_u = R_{PAMPA}/f_u$. The blue dotted line is $R_{PAMPA}/f_u = 1$

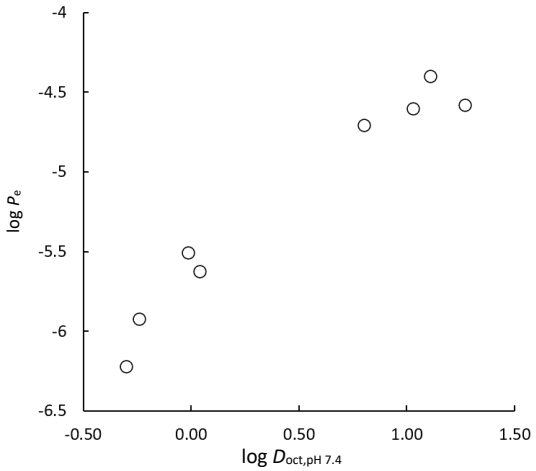


Figure 4. Correlation between log $D_{oct,\ pH\ 7.4}$ and log P_e

In contrast, for low-permeability drugs, interference from the unstirred water layer is negligible. In addition, as shown in Table 3, R_{PAMPA}/f_u tended to be slightly higher than unity. Therefore, to focus on FFT, the membrane permeation of the low-permeability drugs is examined in the following sections.

Concentration dependency of parallel artificial membrane permeation assay membrane permeation

For the low-permeability drugs, there are at least two possible reasons for the slight positive deviation from FFT: (i) albumin-mediated uptake, and (ii) concentration dependence of P_e. To explore the possibility of (ii), the effect of drug concentration on P_e was investigated (Table 4). The lowest concentration was set close to the unbound (free) drug concentration in the presence of 4 % BSA (= $f_u C_D(0)$) at $C_D(0)$ = 100 µM. The P_e values increased as drug concentration decreased.

For entacapone and meloxicam, the concentration-dependent decrease in P_e in the absence of BSA was similar to the R_{PAMPA}/f_u value, suggesting that the deviation from FFT is likely due to the concentration dependence of passive permeation of unbound drug molecules. Passive transport of a drug is usually

considered to be concentration-independent. However, some weak base drugs have previously been reported to show concentration-dependent permeability in PAMPA due to ion-pair transport with anionic phospholipids [37,38]. By contrast, in acidic drugs, such ion-pair-mediated transport cannot occur because both the drug and the phospholipid carry negative charges. For acidic drugs, the formation of dimers or aggregates in the donor solution at higher concentrations may underlie the observed concentration dependence [39]. This possibility remains to be investigated.

Table 4. Concentration dependency of PAMPA permeability for low-permeability drugs in the absence of BSA[a]

Drug	C / μM	P_e / 10^6 cm s^{-1}	P_e ratio (*vs.* P_e at 100 μM)
Entacapone	4	1.5 ± 0.1	1.4
	10	1.3 ± 0.1	1.3
	20	1.2 ± 0.0	1.2
	100	1.0 ± 0.1	1.0
Meloxicam	2	2.4 ± 0.0	1.3
	4	2.1 ± 0.1	1.1
	21	2.0 ± 0.0	1.1
	100	1.8 ± 0.0	1.0
Rosuvastatin	14	0.60 ± 0.01	1.1
	28	0.52 ± 0.10	1.0
	93	0.52 ± 0.02	1.0
Tolbutamide	12.5	3.3 ± 0.3	1.1
	25	3.2 ± 0.2	1.0
	100	3.1 ± 0.4	1.0

[a]PAMPA membrane: 10 % SLP-white/decane, 5 μL.

In contrast, for rosuvastatin, the deviation from FFT was not fully attributable to concentration-dependent passive permeation. Therefore, the possibility of albumin-mediated uptake remains.

Effect of phospholipid composition

The effect of phospholipid composition on P_e was investigated for rosuvastatin and tolbutamide because little concentration dependence in their P_e was observed. Since the purified phosphatidylcholine (PC-98N) could not be dissolved in decane at 10 %, its concentration was reduced to 5 %, and the same concentration was used for the other phospholipids for comparison. In R_{PAMPA}, a small but statistically significant difference was observed among the phospholipids, possibly indicating an interaction between BSA and the PAMPA membrane (Table 5).

Table 5. Effect of phospholipid composition on PAMPA permeability (Mean ± S.D., N = 6)

Drug	Phospholipid[a]	P_e / 10^6 cm s^{-1} [b]		R_{PAMPA}	R_{PAMPA} / f_u	R_{PAMPA} p-value[c]
		-BSA	+BSA			
Rosuvastatin	SLP-white	0.31 ± 0.01	0.10 ± 0.00	0.32 ± 0.01	1.9	
	SLP-PC 70	0.27 ± 0.01	0.10 ± 0.01	0.37 ± 0.03	2.2	0.0085[d]
Tolbutamide	SLP-white	1.67 ± 0.05	0.34 ± 0.01	0.20 ± 0.01	1.1	
	SLP-PC 70	1.94 ± 0.08	0.48 ± 0.02	0.25 ± 0.01	1.4	0.001[e]
	PC-98N	0.79 ± 0.03	0.18 ± 0.02	0.23 ± 0.02	1.3	0.041[e]

[a]PAMPA membrane: 5 % phospholipid/decane, 10 μL. See Table 2 for phospholipid composition. No permeation of rosuvastatin was detected when PC-98N was used; [b]Drug concentration: 0.1 mM; [c]Calculated using EZR software [40]; [d]Student's *t*-test *vs.* SLP-white [e]Tukey's test vs. SLP-white

Limitations of this study

The results obtained in the present PAMPA study do not negate the possibility of albumin-mediated uptake in passive diffusion across cellular membranes. The PAMPA membrane consists of phospholipids dissolved in an organic solvent, and therefore its properties may differ from those of the phospholipid bilayer in cells. PAMPA may not be suitable for evaluating albumin-mediated uptake.

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Conclusions

In conclusion, the PAMPA permeation of the model drugs was substantially reduced by the addition of BSA, showing good consistency with FFT. The deviation from FFT in PAMPA is minimal relative to that in wt-HEK293 cells [8]. In the present study, the slight deviations from FFT observed for entacapone and meloxicam were likely due to concentration-dependent passive permeation. In contrast, the slight deviation from FFT for rosuvastatin may be attributed to albumin-phospholipid interactions.

Supplementary material

Additional data are available at https://pub.iapchem.org/ojs/index.php/admet/article/view/3700, or from the corresponding author on request.

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Data availability: *The datasets generated and/or analysed during the current study are available from the corresponding author on request.*

References

[1] S.G. Summerfield, J.W.T. Yates, D.A. Fairman. Free Drug Theory - No Longer Just a Hypothesis? *Pharmaceutical Research* **39** (2022) 213-222. https://doi.org/10.1007/s11095-022-03172-7

[2] I. Loryan, A. Reichel, B. Feng, C. Bundgaard, C. Shaffer, C. Kalvass, D. Bednarczyk, D. Morrison, D. Lesuisse, E. Hoppe, G.C. Terstappen, H. Fischer, L. Di, N. Colclough, S. Summerfield, S.T. Buckley, T.S. Maurer, M. Fridén. Unbound Brain-to-Plasma Partition Coefficient, $K_{p,uu,brain}$ - a Game Changing Parameter for CNS Drug Discovery and Development. *Pharmaceutical Research* **39** (2022) 1321-1341. https://doi.org/10.1007/s11095-022-03246-6

[3] P.J.H. Webborn, K. Beaumont, I.J. Martin, D.A. Smith. Free Drug Concepts: A Lingering Problem in Drug Discovery. *Journal of Medicinal Chemistry* **68** (2025) 6850-6856. https://doi.org/10.1021/acs.jmedchem.5c00725

[4] J.A. Schulz, D.M. Stresser, J.C. Kalvass. Plasma protein-mediated uptake and contradictions to the free drug hypothesis: a critical review. *Drug Metabolism Reviews* **55** (2023) 205-238. https://doi.org/10.1080/03602532.2023.2195133

[5] T. Baba, A. Touchi, K. Ito, Y. Yamaguchi, Y. Yamazoe, Y. Ohno, Y. Sugiyama. Effects of serum albumin and liver cytosol on CYP2C9-and CYP3A4-mediated drug metabolism. *Drug Metabolism and Pharmacokinetics* **17** (2002) 522-531. https://doi.org/10.2133/dmpk.17.522

[6] M. Trunzer, J. Teigão, F. Huth, B. Poller, S. Desrayaud, R. Rodríguez-Pérez, B. Faller. Improving in vitro-in vivo extrapolation of clearance using rat liver microsomes for highly plasma protein-bound molecules. *Drug Metabolism and Disposition* **52** (2024) 345-354. https://doi.org/10.1124/dmd.123.001597

[7] S.P.F. Tan, A. Tillmann, S.J. Murby, A. Rostami-Hodjegan, D. Scotcher, A. Galetin. Albumin-Mediated Drug Uptake by Organic Anion Transporter 1/3 Is Real: Implications for the Prediction of Active Renal Secretion Clearance. *Molecular Pharmaceutics* **21** (2024) 4603-4617. https://doi.org/10.1021/acs.molpharmaceut.4c00504

[8] Y. Bi, S. Ryu, D.A. Tess, A.D. Rodrigues, M.V. Varma. Effect of human plasma on hepatic uptake of organic anion-transporting polypeptide 1B substrates: studies using transfected cells and primary human hepatocytes. *Drug Metabolism and Disposition* **49** (2021) 72-83. https://doi.org/10.1124/dmd.120.000134

[9] Z. Yan, L. Ma, N. Hwang, J. Huang, J.R. Kenny, C.E. Hop. Using the dynamic well-stirred model to extrapolate hepatic clearance of organic anion-transporting polypeptide transporter substrates

without assuming albumin-mediated hepatic drug uptake. *Drug Metabolism and Disposition* **52** (2024) 548-554. https://doi.org/10.1124/dmd.124.001645

[10] Z. Yan, L. Ma, P. Carione, J. Huang, N. Hwang, J.R. Kenny, C.E. Hop. Introducing the dynamic well-stirred model for predicting hepatic clearance and extraction ratio. *Journal of Pharmaceutical Sciences* **113** (2024) 1094-1112. https://doi.org/10.1016/j.xphs.2023.12.020

[11] Y. Fukuchi, K. Toshimoto, T. Mori, K. Kakimoto, Y. Tobe, T. Sawada, R. Asaumi, T. Iwata, Y. Hashimoto, K. Nunoya. Analysis of nonlinear pharmacokinetics of a highly albumin-bound compound: contribution of albumin-mediated hepatic uptake mechanism. *Journal of Pharmaceutical Sciences* **106** (2017) 2704-2714. https://doi.org/10.1016/j.xphs.2017.04.052

[12] N. Li, A. Badrinarayanan, K. Ishida, X. Li, J. Roberts, S. Wang, M. Hayashi, A. Gupta. Albumin-Mediated Uptake Improves Human Clearance Prediction for Hepatic Uptake Transporter Substrates Aiding a Mechanistic In Vitro-In Vivo Extrapolation (IVIVE) Strategy in Discovery Research. *The AAPS Journal* **23** (2021) 1. https://doi.org/10.1208/s12248-020-00528-y

[13] C. Tang, Y. Lin, A.D. Rodrigues, J.H. Lin. Effect of albumin on phenytoin and tolbutamide metabolism in human liver microsomes: an impact more than protein binding. *Drug Metabolism and Disposition* **30** (2002) 648-654. https://doi.org/10.1124/dmd.30.6.648

[14] R. Weisiger, J. Gollan, R. Ockner. Receptor for Albumin on the Liver Cell Surface May Mediate Uptake of Fatty Acids and Other Albumin-Bound Substances. *Science* **211** (1981) 1048-1051. https://doi.org/10.1126/science.6258226

[15] R.K. Ockner, R.A. Weisiger, J.L. Gollan. Hepatic uptake of albumin-bound substances: albumin receptor concept. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **245** (1983) G13-G18. https://doi.org/10.1152/ajpgi.1983.245.1.G13

[16] S. Miyauchi, M. Masuda, S.-J. Kim, Y. Tanaka, K.-R. Lee, S. Iwakado, M. Nemoto, S. Sasaki, K. Shimono, Y. Tanaka. The phenomenon of albumin-mediated hepatic uptake of organic anion transport polypeptide substrates: prediction of the in vivo uptake clearance from the in vitro uptake by isolated hepatocytes using a facilitated-dissociation model. *Drug Metabolism and Disposition* **46** (2018) 259-267. https://doi.org/10.1124/dmd.117.077115

[17] M.S.C. Abreu, L.M.B.B. Estronca, M.J. Moreno, W.L.C. Vaz. Binding of a fluorescent lipid amphiphile to albumin and its transfer to lipid bilayer membranes. *Biophysical Journal* **84** (2003) 386-399. https://doi.org/10.1016/S0006-3495(03)74859-0

[18] K. Sugano, M. Kansy, P. Artursson, A. Avdeef, S. Bendels, L. Di, G.F. Ecker, B. Faller, H. Fischer, G. Gerebtzoff, H. Lennernaes, F. Senner. Coexistence of passive and carrier-mediated processes in drug transport. *Nature Reviews Drug Discovery* **9** (2010). https://doi.org/10.1038/nrd3187

[19] M. Kansy, F. Senner, K. Gubernator. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. *Journal of Medicinal Chemistry* **41** (1998) 1007-1010. https://doi.org/10.1021/jm970530e

[20] A. Avdeef, S. Bendels, L. Di, B. Faller, M. Kansy, K. Sugano, Y. Yamauchi. PAMPA - critical factors for better predictions of absorption. *Journal of Pharmaceutical Sciences* **96** (2007) 2893-2909. https://doi.org/10.1002/jps.21068

[21] B. Faller, H.P. Grimm, F. Loeuillet-Ritzler, S. Arnold, X. Briand. High-throughput lipophilicity measurement with immobilized artificial membranes. *Journal of Medicinal Chemistry* **48** (2005) 2571-2576. https://doi.org/10.1021/jm049377w

[22] A. Bujard, M. Sol, P.-A. Carrupt, S. Martel. Predicting both passive intestinal absorption and the dissociation constant toward albumin using the PAMPA technique. *European Journal of Pharmaceutical Sciences* **63** (2014) 36-44. https://doi.org/10.1016/j.ejps.2014.06.025

[23] A. Bujard, C. Petit, P.-A. Carrupt, S. Rudaz, J. Schappler. HDM-PAMPA to predict gastrointestinal absorption, binding percentage, equilibrium and kinetics constants with human serum albumin and using 2 end-point measurements. *European Journal of Pharmaceutical Sciences* **97** (2017) 143-150. https://doi.org/10.1016/j.ejps.2016.11.001

(cc) BY

[24] S. Yamauchi, K. Sugano. Permeation characteristics of tetracyclines in parallel artificial membrane permeation assay. *ADMET and DMPK* **7** (2019) 151-160. https://doi.org/10.5599/admet.657

[25] Viatris Pharmaceuticals Japan Inc. *Atorvastatin Drug Product Information*. https://www.viatris-e-channel.com/viatris-products/di/detail/assetfile/Lipitor_Tab_IF.pdf (accessed March 22, 2016) in Japanese

[26] Y. Higashiguchi, S. Ishida, S. Lee, B. Sinko, K. Box, K. Sugano. Predicting Food Effect On Oral Drug Absorption For Solubility-Epithelial Membrane Permeation-Limited Cases With Bile Micelle Solubilization. *Pharmaceutical Research* **42** (2025) 1837-1855. https://doi.org/10.1007/s11095-025-03947-8

[27] Actelion Pharmaceuticals Ltd, Actelion Ltd. Bosentan Drug Product Information. https://pins.japic.or.jp/pdf/medical_interview/IF00003694.pdf (accessed July 22, 2025) in Japanese

[28] A. Avdeef. Absorption and Drug Development: Solubility, Permeability, and Charge State, John Wiley & Sons, 2012. https://doi.org/10.1002/047145026X

[29] Kowa Company, Ltd. Pitavastatin Drug Product Information. https://medical.kowa.co.jp/asset/item/50/1-pi_124.pdf (accessed December 5, 2010) in Japanese

[30] J. Savolainen, M. Forsberg, H. Taipale, P.T. Männistö, K. Järvinen, J. Gynther, P. Jarho, T. Järvinen. Effects of aqueous solubility and dissolution characteristics on oral bioavailability of entacapone. *Drug Development Research* **49** (2000) 238-244. https://doi.org/10.1002/1098-2299(200004)49:4%3C238::AID-DDR2%3E3.0.CO;2-V

[31] Novartis Pharma Co., *Ltd. Entacapone Drug Product Information*. https://www.gifu-upharm.jp/di/mdoc/iform/2g/i1307022310.pdf (accessed September 2, 2021)

[32] P. Luger, K. Daneck, W. Engel, G. Trummlitz, K. Wagner. Structure and physicochemical properties of meloxicam, a new NSAID. *European Journal of Pharmaceutical Sciences* **4** (1996) 175-187. https://doi.org/10.1016/0928-0987(95)00046-1

[33] AstraZeneca, *Rosuvastatin Drug Product Information*. https://med.astrazeneca.co.jp/product/CRS.html (accessed September 7, 2026)

[34] O. Tsinman, K. Tsinman, N. Sun, A. Avdeef. Physicochemical Selectivity of the BBB Microenvironment Governing Passive Diffusion- Matching with a Porcine Brain Lipid Extract Artificial Membrane Permeability Model. *Pharmaceutical Research* **28** (2011) 337-363. https://doi.org/10.1007/s11095-010-0280-x

[35] A. Avdeef, P.E. Nielsen, O. Tsinman. PAMPA - A drug absorption in vitro model: 11. Matching the in vivo unstirred water layer thickness by individual-well stirring in microtitre plates. *European Journal of Pharmaceutical Sciences* **22** (2004) 365-374. https://doi.org/10.1016/j.ejps.2004.04.009

[36] E. Barta, S. Sideman, J.B. Bassingthwaighte. Facilitated Diffusion and Membrane Permeation of Fatty Acid in Albumin Solutions. *Annals of Biomedical Engineering* **28** (2000) 331-345. https://doi.org/10.1114/1.274

[37] K. Sugano, Y. Nabuchi, M. Machida, Y. Asoh. Permeation characteristics of a hydrophilic basic compound across a bio-mimetic artificial membrane. *International Journal of Pharmaceutics* **275** (2004). https://doi.org/10.1016/j.ijpharm.2004.02.010

[38] Z.S. Teksin, K. Hom, A. Balakrishnan, J.E. Polli. Ion pair-mediated transport of metoprolol across a three lipid-component PAMPA system. *Journal of Controlled Release* **116** (2006) 50-57. https://doi.org/10.1016/j.jconrel.2006.08.020

[39] A. Avdeef. Anomalous solubility behavior of several acidic drugs. *ADMET and DMPK* **2** (2014) 33-42. https://doi.org/10.5599/admet.2.1.30

[40] Y. Kanda. Investigation of the freely available easy-to-use software 'EZR'for medical statistics. *Bone Marrow Transplantation* **48** (2013) 452-458. https://doi.org/10.1038/bmt.2012.244

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