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Original article

A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential

Chengyue Zhu *, Lan Jiang, Teng-Man Chen, Kin-Kai Hwang

Aventis Pharmaceuticals Inc., Bridgewater, NJ 08807, USA

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Abstract

Artificial membrane permeability measurement is a potentially high throughput and low cost alternative for in vitro assessment of drug absorption potential. It will be an ideal screening/profiling tool in the lead generation program of drug discovery research if it is proven to be generally applicable for classifying drug absorption potential and is advantageous over other in vitro or in silico methods. This study provides an in-depth evaluation of the method in close comparison to Caco-2, Log D, Log P, polar surface area (PSA), and quantitative structure-property relationship (QSPR) predictions using a large and diverse compound set. It showed that the accuracy of using artificial membrane permeability in assessing drug absorption is comparable to Caco-2, but significantly better than Log P, Log D, PSA, and QSPR predictions. This study also explored the artificial membrane composition by adopting a hydrophilic filter membrane for artificial membrane (lecithin–dodecane) support. The use of hydrophilic filter membrane increased the rate of permeation significantly and reduced the transport time to 2 h or less as compared with over 10 h when a hydrophobic filter membrane is used. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Artificial membrane; Permeability; High throughput screening; Human drug absorption; Caco-2; Lipophilicity; Log P; Log D; Polar surface area

1. Introduction

It has been widely recognised in today's drug discovery research that biopharmaceutical properties must be addressed early in order to generate better quality chemical libraries, leads, and early candidates with enhanced drug-like profile and success rate. One key biopharmaceutical component is oral availability, as oral delivery is the most preferred method for drug administration. In drug discovery research, various in vitro measured or computationally calculated properties have been used to assess the oral absorption potential for drug candidates with various degrees of success. These properties include lipophilicity as measured by traditional octanol–water partition coefficient [1–4], lipidphilicity as measured by liposome–water partitioning [5–7] or immobilised artificial membrane (IAM) chromatography [8–10], hydrogen bonding potential as measured by ΔLog P [11] or molecular polar surface area (PSA) [12,13], and membrane permeability across Caco-2 cell monolayer [14–21]. Among them, Caco-2 monolayer is the most advanced in vitro model because it mimics most transport pathways in the GI tract and has gained broad acceptance as the surrogate marker for estimating in vivo drug absorption potential.

As combinatorial chemistry and high throughput biological screening have been generating increasing number of hits and leads in recent years, higher throughput methods for evaluating biopharmaceutical properties are greatly desired. Although a pure computational (in silico) approach of predicting oral absorption potential is attractive [22,23], its accuracy is less than satisfactory at present. The accuracy of an in silico prediction, which is usually based on a quantitative structure-property relationship (QSPR) model, is also limited by the size, quality, and representativeness of the training set used to derive the model. Therefore, high throughput experimental methods for determining relevant properties for assessing drug absorption potential is still of great importance. Caco-2 membrane permeability assay is widely utilised in the pharmaceutical industry for in

* Correspondence and reprints.
E-mail address: cheng.zhu@aventis.com (C. Zhu).

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vitro assessment of drug absorption potential, but its use as a high throughput tool is limited by the long membrane growth cycle and high implementation cost. Artificial membrane has been investigated before as an alternative model for GI membrane [24–26]. But the parallel artificial membrane permeability assay (PAMPA), first introduced by Kansy et al. [27–30], offered a potentially high throughput approach to measure artificial membrane permeability and to assess drug absorption potential. Since the majority of drugs are absorbed primarily or partially through passive transport, the rate of permeation through a simple artificial membrane, which mimics passive transcellular transport, is likely to provide a good indication of a drug's absorption potential.

The primary goal of this study was to provide an in-depth evaluation of the artificial membrane permeability assay for its general applicability in assessing drug absorption potential and to show its advantages over other in vitro or in silico alternatives. In this study, the correlation of artificial membrane permeability to human fraction absorption was closely compared with those of Caco-2 permeability, lipophilicity (Log P and Log D), PSA, as well as QSPR predicted permeability coefficient using a diverse set of commercial drugs. Since the property of an artificial membrane and its usefulness as a GI membrane model is critically determined by its composition, this study explored the use of a hydrophilic filter membrane (rather than hydrophobic membrane used by others) as the supporting material for phospholipid membrane.

2. Materials and methods

2.1. Materials

Commercially available pharmaceutical compounds were purchased from Sigma/RBI (St. Louis, MO). Egg lecithin (phosphatidylcoline (PC)) was also purchased from Sigma/RBI. Other reagents were purchased from Aldrich (St. Louis, MO) and Fisher Scientific (Fairlawn, NJ).

2.2. Artificial membrane permeability assay

In an artificial membrane permeability experiment, a 96-well filtration plate (hydrophilic PVDF, low-protein binding, Millipore, Bedford, MA) was used for the artificial membrane support and as the receiver plate. The filter material in each well of the filtration plate was wetted with 5 μL of the artificial membrane solution, which consisted of 1% egg lecithin in *n*-dodecane. Then the filtration plate was securely placed on top of a donor plate (Dynex, Middlesex, UK) which was pre-filled with donor solutions (100–200 μM drug solution in phosphate buffer, pH 5.5 or 7.4) in each well. Equal volumes of blank receiving solution were quickly added to the wells of the filtration plate. The stacked donor–receiver plates were incubated at room temperature for 2 h with gentle circular shaking. After incubation, the receiving solutions were sampled and assayed against the initial donor solution concentrations either by HPLC or by a multi-wavelength UV plate reader (SpectroMax190, Molecular Devices, Sunnyvale, CA) at 240–300 nm. The artificial membrane permeability may be expressed either as percent transport (%T) or as apparent permeability coefficient P_{app}.

$$\%T = 100 \times \frac{A_R \cdot V_R}{A_{D0} \cdot V_D} \quad (1)$$

where A_{D0} and A_R are the HPLC peak areas (or UV absorbances) of the initial donor solution and the receiving solution after incubation, V_R and V_D are the volumes of the receiving and donor solutions.

The %T is related to P_{app} based on the following equation:

$$P_{app} = \frac{V_D \cdot V_R}{(V_D + V_R)S \cdot t} \ln\left[\frac{100 \cdot V_D}{100 \cdot V_D - \%T(V_D + V_R)}\right] \quad (2)$$

where S is the surface area of the artificial membrane and t is the incubation time. Eq. (2) is derived from the integration of the Fick's law under the assumption that transport equilibrium is not attained (i.e. $C_D > C_R$),

$$J = \frac{dm}{dt} = P_{app} \cdot S(C_D - C_R) \quad (3)$$

where J is the flux (rate of mass transport) across the membrane, C_D and C_R are the drug concentrations at the donor side and receiver side, respectively. For most drugs, transport equilibration was not attained within 2 h of incubation. Only for very few exceptionally well permeable drugs that fast equilibration may become a possibility. However, the P_{app} values for these highly permeable drugs will be rather high (to reflect near 50% transport) and their status of high permeability classification will not be affected.

2.3. HPLC analysis for artificial membrane permeability

The conditions used for HPLC analyses were as follows. Column: YMC basic 4.6 × 50 mm, 3 μm (YMC, Wilmington, NC). Mobile phase: A—water–0.1% formic acid, B—95% acetonitrile–5% water–0.1% formic acid. Run time: 6 min gradient run for each sample (Waters Alliance 2790 system, Waters Corp., Milford, MA). Flow rate: 1.5 mL min^{-1}; Injection volume: 40 μL. Detection: UV 235 and 254 nm (Waters 966 PDA detector).

2.4. Determination of octanol–buffer distribution coefficient (Log D)

Log D values were determined using a micro shake-flask method. In a micro shake-flask experiment, ca. 0.2 mg of the sample was first mixed with 50–100 μL of *n*-octanol (water saturated) in a 4 mL glass vial, then 2 mL of an aqueous buffer (50 mM phosphate buffer, pH 7.4) was added. The sample vial was placed on a vortex shaker and shaken for 4 h at 180 rpm. After equilibration, the aqueous and octanol phases were sampled and assayed by HPLC to determine the Log D value. The HPLC conditions were the same as those for artificial membrane permeability assay.

2.5. Calculation of polar surface area (PSA)

PSA calculations (non-dynamic) from molecular structures were performed using the SAVOL/SYBYL software [31] (Tripos, Inc. St. Louis, MO) on a Unix workstation via a web interface developed internally. A rapid calculation method for three dimensional molecular structures developed by Pealrman [32] was utilised in the SAVOL software.

2.6. Prediction of human intestine permeability in silico

A simple in-silico approach based on in-house calculated PSA and Lennernas's QSPR model [33] was used in this study to predict the human intestine permeability.

3. Results and discussion

A set of 93 commercial drugs with known human fraction absorption (F_a) data and/or literature Caco-2 P_{eff} data (Table 1) were selected for the evaluation of artificial membrane permeability assay. Most of these compounds were known to be absorbed in human through passive or partially passive transport mechanisms across GI membranes.

3.1. Choice of artificial membrane

Artificial membrane has been shown to be a promising model for biological membranes [24–26]. The adoption of a 96-well filtration plate by Kansy [27] first allowed the artificial membrane permeability assay to be performed in parallel and high throughput mode. Although the filter membrane was used as the support for the impregnated phospholipid solution, the intended biological mimic, its physicochemical property does have profound impact on the actual drug permeability observed. Kansy et al. used a hydrophobic filtration membrane to support the egg lecithin solution in *n*-dodecane. Sugano et al. [28] explored the use of a phospholipid mixture (containing PC, phosphatidyl-serine, phosphatidylethanoamine, and cholesterol) in 1,7-octadiene on the same hydrophobic filter membrane. Faller et al. [29] explored the use of a hydrophobic polycarbonate culture membrane (in 96-well format) coated with hexadecane alone. Each strategy appeared to work well and showed promising correlation between artificial membrane permeability and human F_a for the compound set studied [27–29]. However, one common disadvantage associated with the use of hydrophobic supporting membrane is the slow permeation rate and long transport period (>10 h) for the permeability experiment. In addition, it was observed in our study that hydrophobic supporting membranes had a tendency to resist the permeation of hydrophilic or ionised compounds (many of them well absorbed in human) too strongly, giving rise to low observed artificial membrane permeability and false negatives for absorption indication. Therefore, this study embraced a hydrophilic filtration membrane as the artificial membrane support. Additional rationale for the use of a hydrophilic filtration membrane is that the hydrophilic filter material would interact less with the phospholipid and hence interfere less with the formation of the lipid bilayer. The imbedded artificial membrane used in this study was PC solution in *n*-dodecane (1%, w/v). Phospholipid mixtures were experimented but not used for two reasons. First, the multi-phospholipid formulation, although theoretically more representative of biological membrane composition, posed greater difficulty in maintaining batch to batch reproducibility during preparation. Second, the lipid mixtures containing phosphatidylserine or phosphatidylethanoamine showed poor membrane integrity when imbedded on a hydrophilic filter membrane and hence over expressed the permeability for many poorly absorbed compounds.

This study presents the first parallel artificial membrane permeability results using a hydrophilic filter-based artificial membrane for a large and diverse set of compounds. One immediately realised advantage of using the hydrophilic filter membrane is the increased permeation rate and dramatically reduced transport time (from over 10 to 2 h).

3.2. Artificial membrane permeability versus human absorption (F_a)

The experimental values of artificial membrane permeability at pH 5.5 and 7.4 are given in Table 1, along with the literature values of F_a, Caco-2 permeability (pH 7.4), and Log P (octanol–water partition coefficient), experimental values of Log D (pH 7.4), computer calculated PSA, and QSPR predicted human intestine permeability. Because the pH of the small intestine, the main organ for oral drug absorption,

Table 1
Permeability, human fraction absorbed, and physicochemical properties for the compounds

	Compound	MW	F_a [a]	Caco-2 [b]	PAMPA P_{app}			Log P [d]	Log D [e]	PSA [f]	HBD [g]	QSPR [h]
			(%)	P_{app}	pH 5.5	pH 7.4	pH 5–7 [c]		pH 7.4			P_{eff}
1	Acebutolol	336	90	0.51	0.2	3.3	3.3	1.61	0.83	92.8	3	0.42
2	Acetaminophen	151	80	–	2.3	3.5	3.5	0.51	0.51	58.1	2	1.91
3	Acetylsalicylic Acid	180	98	2.4	3.2	3.8	3.8	1.19	−2.25	67.3	1	2.95
4	Acyclovir	225	21	0.25	0.0	0.0	0.0	−1.74	−1.86	133.5	4	0.08
5	Alprenolol	249	94	25.3	1.4	15.1	15.1	3.1	1.38	43.7	2	2.75
6	Amiloride	229	50	–	0.0	0.0	0.0	–	−0.86	156.9	8	0.00
7	Amoxicillin	365	93	0.8	0.0	1.5	1.5	0.76	−1.69	148	5	0.03
8	Amphotericin B	924	5	–	0.0	0.0	0.0	–	−1.52	55.5	13	0.00
9	Antipyrine	188	100	28.2	20.1	13.2	20.1	0.38	0.34	29.4	0	14.79
10	Atenolol	266	52	0.2	0.1	0.0	0.1	0.16	−1.29	94.6	4	0.20
11	Bretylium tosylate	414	22	–	0.0	0.0	0.0	–	–	–	–	–
12	Bromocriptine	654	28	–	1.3	0.0	1.3	6.62	4.61	107.2	3	0.29
13	Bumetanide	364	100	–	4.6	0.3	4.6	4.06	−0.11	124	4	0.10
14	Bupropion	239	91	–	48.3	14.1	48.3	3.21	2.61	26.4	1	8.32
15	Caffeine	194	100	30.8	20.6	10.8	20.6	−0.07	0.02	59.2	0	6.92
16	Captopril	217	71	–	4.4	19.1	19.1	1.02	−2.00	64	1	3.16
17	Carbamazepine	236	100	–	12.0	11.3	12.0	2.45	1.78	48.6	2	2.45
18	Cefadroxil	363	100	–	0.0	1.0	1.0	−0.09	−1.77	148.3	5	0.03
19	Ceftriaxone	554	1	–	0.1	0.0	0.1	–	−1.20	235.3	5	0.00
20	Cephalexin	347	96	0.5	0.0	0.1	0.1	0.65		124.3	4	0.10
21	Chloramphenicol	323	90	20.6	6.7	1.7	6.7	1.14	1.14	119.6	3	0.21
22	Chlorothiazide	295	13	0.15	0.2	1.3	1.3	−0.24	−0.05	137	3	0.13
23	Chlorpromazine	318	100	19.9	11.8	4.0	11.8	5.35	3.38	10.4	0	23.99
24	Cimetidine	252	93	0.74	0.0	0.0	0.0	0.4	0.35	93.4	3	0.41
25	Clofibrate	466	100	–	0.4	0.3	0.4	3.65	3.39	36.5	0	12.30
26	Clonidine	242	96	30.1	20.0	14.0	20.0	1.37	0.62	42.2	2	2.88
27	Clozapine	230	100	–	22.2	28.1	28.1	1.59	0.84	42.2	2	2.88
28	Corticosterone	346	100	54.5	39.0	22.1	39.0	1.86	1.89	76	2	1.23
29	Coumarin	148	100	–	22.9	22.1	22.9	1.41	1.39	34.1	0	13.18
30	Cromolyn	468	1	–	0.0	0.0	0.0	1.95	−1.15	185.9	3	0.04
31	Cyclosporine	1202	35	0.9	0.1	0.3	0.3	–	2.31		5	0.00
32	Desferrioxamine	560	2	–	0.0	0.0	0.0	–	−0.11		7	0.00
33	Desipramine	266	100	21.6	9.4	14.6	14.6	4.9	1.28	19.2	1	10.00
34	Dexamethasone	392	98	12.2	6.8	8.1	8.1	1.83	1.83	92.1	3	0.42
35	Diclofenac	318	100	–	10.7	12.5	12.5	4.4	1.15	42.4	2	2.88
36	Diltiazem	414	92	29.8	10.6	18.5	18.5	2.7	2.06	60.3	0	6.76
37	Doxorubicin	543	5	0.16	0.3	0.5	0.5	1.27	−0.16	205.7	7	0.00
38	Enalapril	376	60	2.31	3.4	0.1	3.4	0.71	−0.90	105.7	2	0.58
39	Erythromycin	733	35	3.73	0.1	0.1	0.1	2.54	1.26	−99	5	0.00
40	Etoposide	588	50	–	0.7	0.4	0.7	1.97	1.82	186.4	3	0.04
41	Flumazenil	303	95	–	4.8	6.0	6.0	1.64	1.21	65.2	0	5.89
42	Fluoxetine	309	80	–	7.4	14.1	14.1	4.05	1.83	25	1	8.51
43	Furosemide	330	60	0.12	0.6	0.6	0.6	2.29	−0.69	132.4	4	0.08
44	Gabapentin	171	50	–	1.2	1.2	1.2	−1.25	−2.00	64.7	3	0.85
45	Griseofulvin	352	27	36.6	7.8	5.3	7.8	2.18	2.18	82.1	0	3.89
46	Guanabenz	231	75	20.9	1.6	17.5	17.5	3.02	1.40	75	4	0.34
47	Hydrochlorothiazide	297	70	0.51	0.1	0.0	0.1	−0.07	−0.12	138.6	4	0.07
48	Hydrocortisone	362	91	14	3.1	3.4	3.4	1.61	1.55	99.4	3	0.35
49	Ibuprofen	206	93	52.5	10.8	6.8	10.8	3.5	0.68	44	1	5.25
50	Imipramine	280	99	14.1	12.9	8.4	12.9	4.8	2.40	9.7	0	24.55
51	Indomethacin	357	100	20.4	6.3	2.4	6.3	4.27	1.00	77.9	1	2.24
52	Ketoconazole	531	75	–	3.3	1.2	3.3	4.34	3.83	67.9	0	5.62
53	Ketoprofen	254	100	–	19.1	16.7	19.1	3.12	−1.51	66.3	1	3.02
54	Ketorolac	255	100	–	5.1	1.4	5.1	1.88	−0.27	70.8	1	2.69
55	Labetalol	328	93	9.31	0.1	4.5	4.5	3.09	1.24	97.4	5	0.10
56	L-Phenylalanine	165	100	–	0.0	0.0	0.0	−1.58	−2.00	68.6	3	0.76
57	Lucifer Yellow	445	0	–	0.0	0.0	0.0	–	–	243.9	7	0.00
58	Melphalan	305	100	–	10.2	5.7	10.2	−0.52	−2.00	70.9	3	0.72
59	Methotrexate	454	47	1.2	0.2	0.1	0.2	−0.46	−2.53	225.9	7	0.00
60	Methylprednisolone	374	82	–	2.6	5.9	5.9	2.2	2.10	97.4	3	0.37

Table 1 (*Continued*)

	Compound	MW	F_a [a]	Caco-2 [b]	PAMPA P_{app}			Log P [d]	Log D [e]	PSA [f]	HBD [g]	QSPR [h]
			(%)	P_{app}	pH 5.5	pH 7.4	pH 5–7 [c]		pH 7.4			P_{eff}
61	Metoprolol	267	95	23.7	1.2	3.5	3.5	1.88	−0.16	58.4	2	1.91
62	Miconazole	416	99	–	0.7	0.0	0.7	6	3.47	25.8	0	16.22
63	Nadolol	309	32	3.88	0.0	0.0	0.0	0.71	0.68	88.8	4	0.24
64	Naproxen	230	98	39.5	22.9	10.6	24.3	3.18	0.23	56.2	1	3.89
65	Nicotine	162	100	19.4	14.8	21.2	21.2	1.32	0.41	19.5	0	19.05
66	Norfloxacin	319	35	–	0.5	0.9	0.9	0.42	−0.81	80.6	2	1.10
67	Penicillin V	350	45	–	1.6	0.1	1.6	2.09	−0.62	110.9	2	0.50
68	Phenytoin	252	90	26.7	7.6	5.1	7.6	2.47	2.26	64.8	2	1.62
69	Pindolol	248	92	16.7	13.1	4.9	13.1	1.75	0.19	62.7	3	0.89
70	Piroxicam	331	100	35.6	8.4	8.2	8.4	1.98	−0.07	111.5	2	0.50
71	Prazosin	383	100	43.6	2.5	13.5	13.5	2.16	1.88	115.7	2	0.45
72	Prednisolone	360	99	–	2.2	5.7	5.7	1.62	1.60	99.9	3	0.35
73	Probenecid	285	90	–	4.0	2.4	4.0	3.21	−0.32	83.4	1	1.95
74	Progesterone	314	93	23.7	0.8	4.0	4.0	3.87	3.48	36.6	0	12.30
75	Propranolol	259	93	41.9	17.1	23.5	23.5	3.56	1.26	43.8	2	2.75
76	Quinidine	324	80	20.4	6.0	10.9	10.9	3.2	2.04	45.9	1	5.01
77	Ranitidine	314	55	0.49	0.0	0.5	0.5	0.27	−0.29	86.8	2	0.93
78	Saccharin	183	100	–	7.1	0.0	7.1	0.91	−0.49	80	1	2.14
79	Salicylic Acid	138	100	41.9	21.5	3.3	21.5	2.26	−1.44	61.3	2	1.78
80	Sotalol	272	95	–	2.9	1.1	2.9	0.24	−1.35	89.9	3	0.45
81	Sulfasalazine	398	12	0.13	0.3	0.1	0.3	4.25	−0.42	152.3	3	0.09
82	Sulpiride	341	36	–	0.2	0.1	0.2	1.31	−1.00	108.7	3	0.28
83	Terazosin	387	91	–	1.7	8.8	8.8	2.29	1.14	113.6	2	0.47
84	Terbutaline	225	68	0.38	0.0	0.1	0.1	0.48	−1.35	80.8	4	0.29
85	Theophyline	180	97	–	3.3	4.8	4.8	−0.25	−0.05	72.5	1	2.57
86	Timolol	316	81	12.8	1.7	5.1	5.1	1.91	0.03	85.2	2	0.95
87	Tranexamic acid	157	55	–	0.0	0.0	–	−1.87	−3.00	69.8	3	0.74
88	Trimethoprim	290	97	–	2.7	5.0	5.0	0.91	0.74	110.8	4	0.13
89	Verapamil	454	98	–	9.7	7.4	9.7	3.79	2.66	77	0	4.47
90	Warfarin	308	97	21.1	10.5	12.3	12.3	2.52	0.64	59.2	1	3.63
91	Zidovudine (AZT)	267	98	6.93	0.6	4.9	4.9	−0.02	−0.58	141.3	2	0.23
92	Zopiclone	388	80	–	3.2	8.9	8.9	0.98	0.50	99.7	0	2.51

[a] Literature F_a values were cited from references [5,13,16,18,20,27,29,31,35–37].
[b] Literature Caco-2 P_{app} (cm s^{-1}, $\times 10^6$) were cited from references [16–18,20,21,36].
[c] Higher value of P_{app} (cm s^{-1}, $\times 10^{-6}$) between pH 5.5 and 7.4, determined by HPLC.
[d] Log P values were cited from reference [38].
[e] Log D values were determined experimentally.
[f] PSA values (A^2) were calculated using SAVOL (reference [31]).
[g] HBD-number of hydrogen bond donor atoms.
[h] Predicted human intestine permeability (cm s^{-1}, $\times 10^4$) based on 5 using PSA values calculated in this study.

varies from 5 to 8 and the permeability of ionisable drugs varies with pH, the higher value of artificial membrane permeability between pH 5.5 and 7.4 was used in this study for the evaluation of correlation to human absorption. Fig. 1 illustrates the correlation of artificial membrane permeability versus human fraction absorbed F_a. As seen in Fig. 1, the artificial membrane permeability correlates to F_a closely through a trendline that consists of a steep slope region ($P_{app} \sim 0.0–3 \times 10^{-6}$ cm s^{-1}) and a plateau region ($P_{app} > 4 \times 10^{-6}$ cm s^{-1}) which is also characteristic in both the Caco-2 versus F_a correlation (Fig. 2) and the intestine permeability versus F_a correlation [33]. This implies that the artificial membrane used in this study does mimic the property of biological membrane to a great extent. The hyperbolic correlation (and its characteristic initial steep-slope) between permeability and F_a may be expressed by following equation:

$$F_a \approx 1 - e^{-k \cdot P_{app}} \quad (4)$$

where k is a constant related to membrane surface area and mean residence time. The presence of the initial steep-slope limits the prediction accuracy for some medium to low absorption drugs, which occurs not only in artificial membrane permeability, but also in Caco-2 and in situ or in vivo permeability models. Nevertheless, artificial membrane permeability does show promising results in classifying compounds into either a good absorption category or a poor or questionable absorption category with high success rate. As seen in Fig. 1, nearly all the compounds (except for griseofulvin) with artificial membrane P_{app} greater than

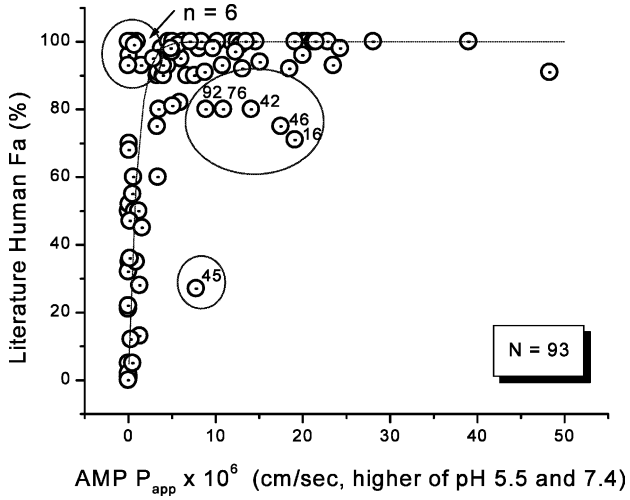

Fig. 1. AMP P_{app} vs. F_a for 93 commercial drugs.

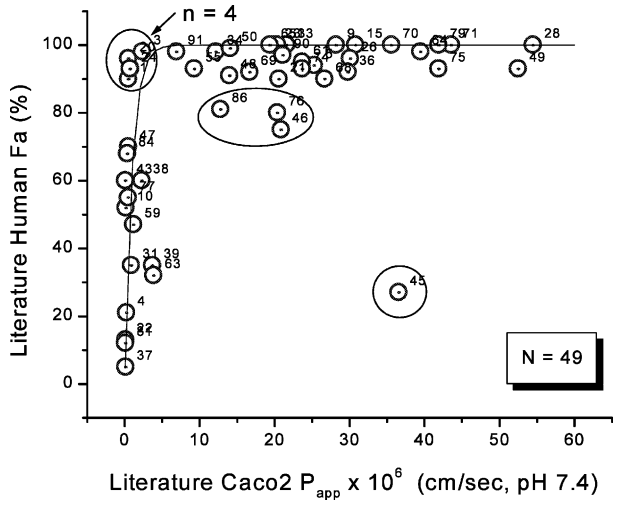

Fig. 2. Caco-2 P_{app} vs. F_a for 49 commercial drugs.

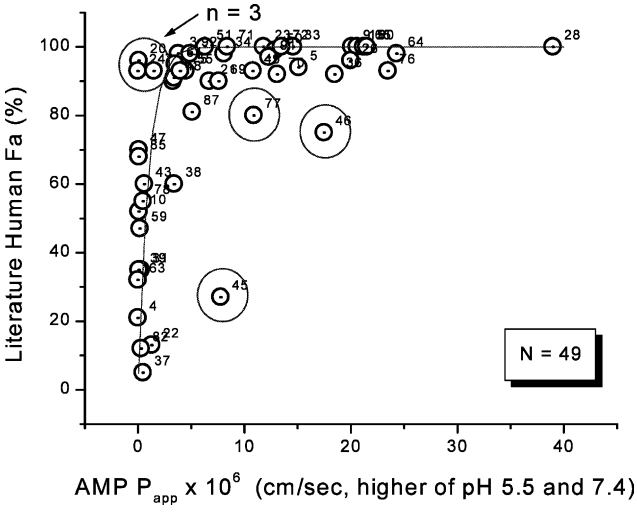

Fig. 3. AMP P_{app} vs. F_a for 49 commercial drugs (same set as in Fig. 2).

3×10^{-6} cm s^{-1} are well absorbed with F_a greater than 80%. Within the steep-slope region, all the poorly or medium absorbed drugs (except for griseofulvin) are found. The success rate of placing highly absorbed drugs in the plateau region and poorly absorbed drugs in the steep-slope region based on artificial membrane P_{app} is close to 90% for the 93 commercial drugs tested. For griseofulvin, studies have shown that its absorption in human varies and is highly formulation dependent. Better absorption (upto 75%) was observed for ultra-micronised preparations [34] as compared with 27% absorption for unmicronised preparations.

The capability of artificial membrane permeability in absorption classification was found to be highly comparable to that of Caco-2 permeability, as evidenced in Figs. 2 and 3 for a common set of 49 compounds (which reliable literature Caco-2 values were available). Artificial membrane permeability and Caco-2 not only showed similar success rate in absorption classification, interestingly, they also shared some common outliers such as amoxicillin, cephalexin, cimetidine, and griseofulvin. Except for griseofulvin, most notable outliers observed in artificial membrane permeability and in Caco-2 are false negatives, i.e. drugs that are well absorbed in humans but poorly permeable through artificial or Caco-2 membrane. Imaginably, these drugs are likely absorbed in humans via a certain degree of transporter assistance, which Caco-2 model failed to mimic.

3.3. Direct correlation between artificial membrane permeability and Caco-2 permeability

Artificial membrane and Caco-2 membrane are different in that the former mimics the passive transcellular route of drug transport only, while Caco-2 to a certain degree mimics additional transport mechanisms such as paracellular transport through tight junction, active transport via transporters, as well as efflux phenomenon induced by P-glycoproteins. Therefore, the correlation between artificial membrane permeability and Caco-2 permeability is not expected to be ideal. However, because the majority of drugs are absorbed through passive (or partially passive) transcellular transport, a certain degree of correlation between artificial membrane permeability and Caco-2 permeability is likely to exist. This has been demonstrated in Fig. 4 for a set of 49 commercial drugs. Good linear correlation between artificial membrane permeability and Caco-2 permeability at pH 7.4 was observed ($R = 0.72$). Better correlation was observed ($R = 0.82$) between the Caco-2 permeability and the higher artificial membrane permeability at pH 5.5 and 7.4 (Fig. 4). The close similarity between artificial membrane permeability and Caco-2 permeability in terms of correlation to F_a, shared false positives and false negatives, and good linear correla-

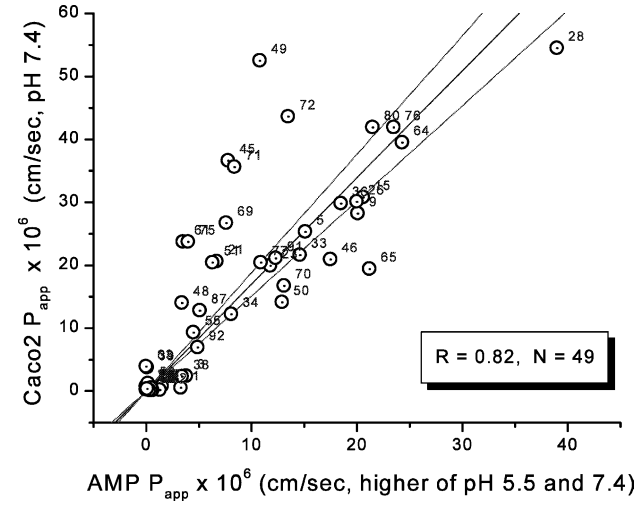

Fig. 4. Correlation between AMP P_{app} and Caco-2 P_{app}.

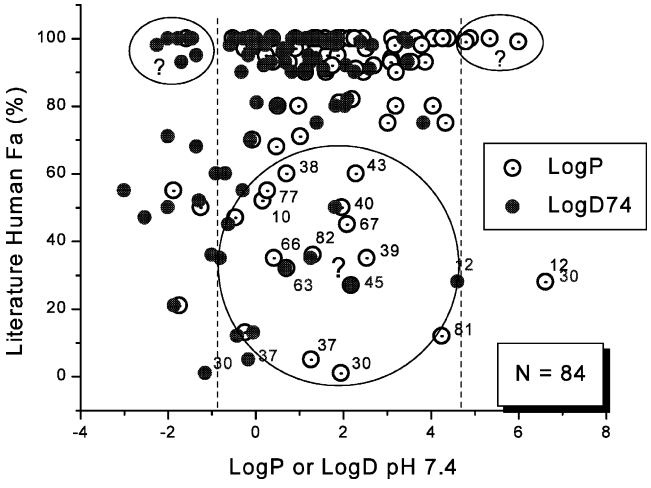

Fig. 5. Log P and Log D (pH 7.4) vs. F_a for 86 commercial drugs.

tion between Caco-2 and artificial membrane permeabilities strongly suggests that passive transcellular transport played an essential role in the absorption of most drugs, thus validating artificial membrane permeability as a simple yet effective model for passive transcellular transport.

3.4. Comparison of artificial membrane permeability with Log P(D), PSA and QSPR predicted permeability

Figs. 5 and 6 illustrate the correlations of Log P(D) versus F_a and PSA versus F_a. As seen in Fig. 5, a general and qualitative observation may be made that Log P between 0.0 and 5 (or Log D between -0.5 and 4.5) will likely relate to good oral absorption. However, false negatives and false positives (circled with question marks), especially the latter, are more frequent than artificial membrane permeability vs. F_a. It is noticed that Log P gave more false positives (Log P within 0–5, but absorption poor) than Log D. This may be due to the fact that Log P reflects only the intrinsic lipophilicity (for neutral molecules), but not the ionisation effect (for ionisable drugs) and the actual lipophilicity at physiological pH. PSA has been shown previously to correlate well with human intestine absorption [12]. However, when PSA is applied to a larger and more diverse compound set, outliers become more frequent, as evident in this study and in Clark's study [13]. Although a general observation may be made from Fig. 6 that PSA less than 120 Å^2 may suggest good absorption, exceptions are numerous as seen in Fig. 6.

Fig. 7 show the relationship between F_a and the predicted intestine permeability based on a simple QSPR model developed by Lennernas et al. [33]:

$$Log\ P_{eff} = -2.546 - 0.011 \cdot PSA - 0.278 \cdot HBD \quad (5)$$

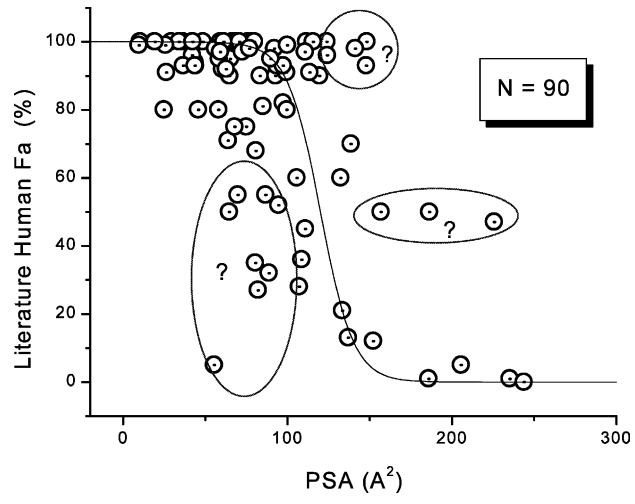

Fig. 6. PSA vs. F_a for 90 commercial drugs.

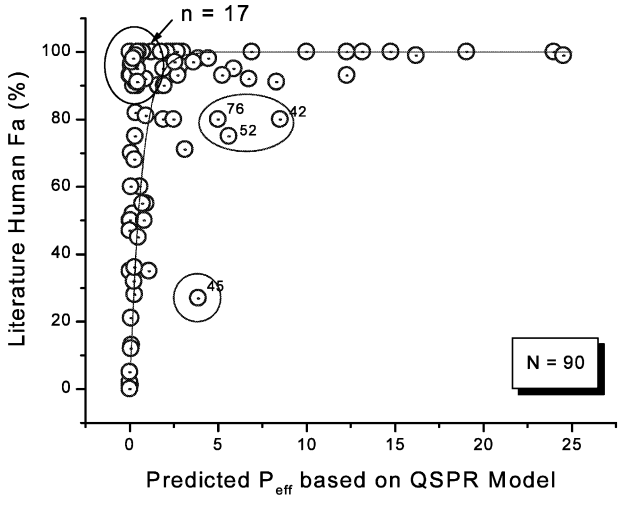

Fig. 7. Calculated intestine P_{eff} based on PSA and number of donor atoms vs. F_a for 90 commercial drugs.

Table 2
Reproducibility of PAMPA transport results: batch-to-batch and day-to-day reproducibility

Compound	Human Abs F_a	AMP P_{app} (pH 7.4) [a]				
		Day 1-batch 1	Day 2-batch 2	Day 3-batch 3	Average P_{app}	Relative deviation (%)
Sulfasalazine	13	0.2	1.4	0.7	0.8	±75
Terbutaline	60	0.9	1.5	2.2	1.5	±40
Chloramphenicol	90	3.2	2.3	3.5	2.9	±21
Diltiazem	92	20.5	21.3	21.8	21.1	±3
Wafarin	93	12.2	14.1	11.8	12.7	±10
Naproxen	94	6.9	11.2	9.4	9.2	±25

[a] P_{app} values determined by UV plate reader.

where P_{eff} is the human intestine permeability (the model was developed using a training set with experimental human intestine permeability data), HBD is the number of hydrogen-bond donor atoms. The calculated P_{eff} results are listed in Table 1.

As seen in Fig. 7, although the predicted P_{eff} correlated to F_a in the similar manner to Caco-2 and artificial membrane permeability, it significantly underestimated more well-absorbed drugs. For the same set of compounds tested, 17 false negatives were found in Fig. 7 for the calculated P_{eff} as compared with six in Fig. 1 for artificial membrane P_{app}. One possible reason for this less than satisfactory correlation is that the QSPR model used was developed using a relatively small training set ($N < 44$) and relatively small number of descriptors [33]. Although intestine permeability has strong dependence on a molecule's PSA and hydrogen bonding potential, as demonstrated by QSPR modelling, the prediction of permeability based on PSA and hydrogen bonding alone may still be of limited applicability.

3.5. Reproducibility of artificial membrane permeability assay

The reproducibility of artificial membrane permeability was evaluated on a day-to-day and batch-to-batch basis using different batches of artificial membrane preparation, as shown in Table 2. The relative standard deviation is generally much smaller than ± 50%. Higher relative standard deviation may be encountered for some very poorly permeable drugs with P_{app} less than 1×10^{-6} cm s^{-1}. For these drugs, their low permeability status (or classification) would not be affected even though their P_{app} carries a 50–100% relative deviation. Although smaller uncertainty level is desirable, the current uncertainty level does not affect the correct differentiation of poorly permeable drugs from medium or well permeable drugs.

3.6. Summary

The value of artificial membrane permeability assay is obvious in many aspects. First, artificial membrane permeability is a better indicator for drug absorption potential than Log P(D), PSA, and current QSPR predictions, and is comparable to Caco-2 permeability, as shown in this study. Second, artificial membrane permeability assay can be high throughput, which is realised through a 96-well parallel format, fast membrane preparation, and the use of UV plate reader and/or fast LC–MS. Third, artificial membrane is potentially more tolerable to a wider pH-range and higher DMSO content (upto 5%) than Caco-2. A wider pH range allows for better coverage of intestine pH range. Higher DMSO content allows for higher sample solubility and less analytical challenge. Finally, artificial membrane permeability assay is low cost and easy to implement. Therefore, it can be adopted in any laboratory that can benefit, e.g. in a combinatorial chemistry lab for library profiling.

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