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Relationship between immobilized artificial membrane chromatographic retention and human oral absorption of structurally diverse drugs

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Abstract

Capacity factors are determined for a set of drugs for which human oral absorption (HOA) data are available, using immobilized artificial membrane (IAM) chromatography. The compound set represented acidic, basic, neutral and amphoteric drugs from various structure classes and having low to high human oral absorption. Effect of mobile phase pH on retention was investigated to determine the optimal condition for better correlation with HOA. The retention (capacity factor, k'_{IAM}) of each drug was measured by reverse phase HPLC using an IAM.PC.DD2 (1 cm × 3 mm i.d., 12 μm) column with an eluent of acetonitrile—0.01 M phosphate buffer at pH 4.5–7.4. The pH dependent k'_{IAM} was in accordance with pH partition theory. Using non-linear regression analysis the obtained $\log k'_{IAM}$ values were compared with published data on HOA in order to establish correlation. The better correlation with HOA was observed when the highest $\log k'_{IAM}$ value selected among pH 4.5–7.4 ($R^2 = 0.8566$) for each drug rather than obtained at more traditional pH 7.4 ($R^2 = 0.7403$). Finally, it was confirmed by Cook's D outlier test that there was no influential observation in the model that affect the relationship between IAM capacity factor and HOA. The assay conditions were optimized and validated to make it suitable for routine analysis and for compound characterization in early discovery where permeability may be an issue.

Keywords: Absorption; Immobilized artificial membrane chromatography; In vitro model; Capacity factor; Correlation

1. Introduction

With the rise of combinatorial chemistry and the ability to produce large collections of individual compound sets, the number of new compounds to be characterized as potential drug candidates has increased. As a result drug design and discovery cannot have pharmacodynamic potency as the sole criterion of optimization but must also take pharmacokinetic behavior into account, absorption and distribution in particular. Many compounds with good therapeutic potential fail to progress beyond the early developmental stages primarily because of insufficient oral bioavailability. Around one-third of development candidates are lost due to inappropriate pharmacokinetic properties (Prentis et al., 1988). The prediction of drug–membrane permeability is important during the lead optimization stage of drug discovery. Several factors, viz. experimental difficulty, high cost and low throughput, involved in screening of lead compounds in animals for oral drug absorption have led to the development of various in vitro prediction model. The drug development process is therefore relied increasingly upon in vitro methods to screen a large number of potentially therapeutic compounds in terms of oral absorption in humans. This will be helpful in an effective lead candidate selection and its optimization in the development phase.

There are two possible pathways for permeation from gastrointestinal (GI) tract following oral administration of a drug: (1) moving across cell membrane—the transcellular route (including passive diffusion, receptor-mediated endocytosis and carrier-mediated uptake) and (2) passive diffusion via tight junction—paracellular route. Most of drugs are absorbed across intestinal mucosa by passive diffusion via transcellular route (Artursson and Karlsson, 1991).

It is well established that passive drug absorption across the gastrointestinal wall is governed by several molecular properties including lipophilicity, molecular size, charge, hydrogen bonding and solubility. Importantly, most of these prop-

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erties are dependent on one another. Solubility and permeability are considered in the framework of the biopharmaceutical classification system as fundamental to define the rate and extent of absorption of the active ingredient of a drug product.

Numerous quantitative structure–permeability relationship (QSPR) studies have unambiguously demonstrated that lipophilicity is a key parameter in predicting and interpreting permeability (van de Waterbeemd and Testa, 1987; Camenisch et al., 1996; Conradi et al., 1996). Lipophilicity as a molecular parameter encodes both polar and hydrophobic intermolecular forces, but it fails to encode some important recognition forces; most notably ionic bonds when expressed by partition coefficient measured in organic solvent/water systems. Ionic bonds are of particular importance because charged forms of some molecules are able to partition into phospholipid bilayer (Testa et al., 1996). Many drugs contain one or more ionizable groups, and their lipophilicity is pH dependent. A study suggests that 62.9% of drugs are ionizable, of which 14.5% are acids, 67.6% are bases and 17.9% are ampholytes of various types (Comer and Tam, 2001). This indicates that selected in vitro model for prediction of oral drug absorption must take ionization into account. The behavior of ionizable drugs in the body is controlled by the interaction of both neutral and ionized form with the biological membranes. The importance of partitioning of ionic and zwitterionic species in biphasic media has been emphasized in connection with their pharmacokinetic and pharmacodynamic behavior, as hydrophobic as well as electrostatic interactions are expected between biological membranes and ionized compounds (Avdeef et al., 1996; Abraham et al., 1997; Pagliara et al., 1998).

A new approach for studying passive absorption of drugs is based on artificial membrane, i.e., liposomes (Beigi et al., 1998), parallel artificial membrane permeability assay (PAMPA) (Kansy et al., 1998), bio-mimetic lipid membrane (Sugano et al., 2001) and immobilized artificial membrane (IAM)-HPLC (Pidgeon et al., 1995). Since artificial membranes provide the amphiphilic microenvironment of biological membranes, they are able to take ionic bonds into account. IAM-HPLC is currently receiving marked interest because it presents the additional advantage of being suitable for high-throughput screening. The chromatographic capacity factors of drug on IAM column were shown to correlate with gastrointestinal absorption (Liu et al., 1995; Pidgeon et al., 1995; Genty et al., 2001; Yen et al., 2005) and permeation across Caco-2 cells (Stewart et al., 1998; Chan et al., 2005). Although there has been much effort to assess IAM chromatography as a predictive tool for drug absorption, much less attention has given into the optimization of the chromatographic conditions for better correlation with percent human oral absorption (HOA) data.

In present work, a set of chemically diverse drugs was applied onto the IAM column and mobile phase system was set in accordance to the physiology of the GI-tract, where a pH gradient is present from the stomach down to the colon. The obtained $\log k'_{\mathrm{IAM}}$ values were compared with published data on HOA in order to establish correlation. The findings of the study and their implications are elaborated in the subsequent sections.

2. Materials and methods

2.1. Materials

The 28 drugs investigated in this study as mentioned in Table 1 were all supplied by Torrent Pharmaceutical Ltd. (Ahmedabad, India) and used without further purification. They were selected to represent diverse physicochemical properties. Citric acid was purchased from Merck (Mumbai, India). Water-soluble drugs were dissolved in water obtained from Milli-Q water purification system from Millipore (Banglore, India) and lipophilic drugs were first dissolved in HPLC grade methanol purchased from Ranbaxy (New Delhi, India) and then diluted with water to get appropriate concentration (∼10 μg/ml). The HPLC mobile phases were 0.01 M Dulbeco's phosphate buffer saline (DPBS) pH 4.5–7.4 from HIMEDIA (Mumbai, India) and HPLC grade acetonitrile from Ranbaxy. The *ortho* phosphoric acid for the pH adjustment was obtained from Merck.

2.2. Chromatographic system

The chromatographic system consisted of CBM-10A system controller, LC-10 ADvp HPLC pump, auto injector SIL-10A (Shimadzu, Kyoto, Japan) equipped with Rheodyne injector (six port valve) module with a 20 μl loop (Rheodyne, Cotati, CA) and SPD-10Avp UV spectrophotometer (Shimadzu, Kyoto, Japan). UV detection was monitored at 220 and 254 nm. The column temperature was maintained at 25 °C using column oven CTO-10A (Shimadzu, Kyoto, Japan). The chromatograms were acquired and processed using the Shimadzu LC-10 software. A commercially available IAM Fast Screen Mini Column (prepared with phosphotidylcholine analogs) was used with the dimensions of 1 cm × 3 mm i.d. obtained through Regis Technologies, Inc. (Morton Grove, IL). The column had a particle diameter of 12 μm and a pore diameter 300 Å. The structure of the chromatographic stationary phase is fully described elsewhere (Yang et al., 1996; Taillardat-Bertschinger et al., 2003). The flow rate was 0.5 ml/min throughout the experimental phase. The mobile phases were filtered through 0.45 μm filters from Millipore. The pH was measured with a pH-meter, μp controlled pH analyzer from LABINDIA (Thane, India). The mobile phases were prepared manually and degassed in an ultrasonic bath prior to use. For all studies, the injection volume was 5 μl of a drug solution.

2.3. Isocratic measurements of $\log k'_{IAM}$

The mobile phase for the isocratic determination of the retention times (t_R), 0, 5, 10, 20 and 30% (v/v) acetonitrile was used. The dead time (t_0) of the system was determined by injecting citric acid solution. The $\log k'_{\mathrm{IAM}}$ values were obtained by $\log[(t_R - t_0)/t_0]$. Hydrophobic drugs had retention times of >30 min when only buffer was used as the mobile phase. Therefore, the $\log k'_{\mathrm{IAM}}$ values referring to the buffer only mobile phase were extrapolated by plotting the $\log k'_{\mathrm{IAM}}$ values and the applied acetonitrile concentration. The intercept of the straight line was used as the extrapolated $\log k'_{\mathrm{IAM}}$ to buffer only mobile

Table 1
%HOA, $\log k'_{IAM}$ and physicochemical values of selected compounds

No.	Compounds	MW[a]	pK_a[b]		$\log k'_{IAM}$ pH 4.5–7.4[c]	Observed %HOA[d]	Calculated %HOA[e]	Cook's D value[f]
			HA	HB+				
1	Acyclovir	225	9.3	2.3	−1.150	17 (13–21)	14	0.0059
2	Amiloride	230		8.7	0.862	50	65	0.0355
3	Atenolol	266		9.6	0.039	44	41	0.0029
4	Bretylium	244			−0.326	23	31	0.0387
5	Carbamazepine	236			1.392	84 (73–96)	78	0.0076
6	Chlorothiazide	295	6.8, 9.5		0.316	49 (36–61)	49	0.0000
7	Chlorpromazine	319		9.3	3.218	96	97	0.0034
8	Chlorthalidone	338	9.4		1.161	65 (60–70)	73	0.0093
9	Ciprofloxacin	331	6	8.8	0.984	67	68	0.0002
10	Dexamethasone	393			1.560	90	81	0.0169
11	Diclofenac	296	4.5		2.534	100	93	0.0118
12	Diltiazem	415		7.7	2.210	90	91	0.0004
13	Famotidine	338		7	0.559	40	56	0.0510
14	Furosemide	331	3.9		1.807	65 (62–67)	85	0.1065
15	Ganciclovir[g]	255	9.4	2.2	−1.560	5	9	0.0300
16	Hydrochlorothiazide	298	7, 9.2		0.584	70	57	0.0325
17	Imipramine	280		9.5	2.600	100	94	0.0098
18	Indomethacin	358	4.5		3.019	100	96	0.0022
19	Ketorolac	255	3.5		1.477	95	80	0.0487
20	Lisinopril	406	1.7, 3.3, 11.1	7	−0.959	25	17	0.0520
21	Metolazone	366	9.7		1.684	64	83	0.0879
22	Naproxen	230	4.2		2.131	100	90	0.0300
23	Norfloxacin	319	6.3	8.8	0.978	65	68	0.0013
24	Propranolol	259		9.5	2.119	95	90	0.0077
25	Ranitidine	314		2.3, 8.2	0.613	50	58	0.0112
26	Terbutaline	225	10.1, 11.2	8.8	0.332	53 (25–80)	50	0.0029
27	Timolol	316		8.8	0.796	90	63	0.1223
28	Verapamil	455		8.9	3.004	90	96	0.0254

[a] Molecular weight.
[b] pK_a values were obtained from Williams and Lemke (2002) and Sugano et al. (2002).
[c] Highest value of $\log k'_{IAM}$ among pH 4.5–7.4, determined by HPLC (values given are average of three determinations).
[d] %HOA values were obtained from previously reported values (Dollery, 1999; Zhao et al., 2001; Sugano et al., 2002). When the %HOA value was reported as a range, the mid-value of the range was used (values in parentheses indicating range).
[e] Calculated %HOA based on Eq. (1) using $\log k'_{IAM\,50\%}$ and slope values from Fig. 3B.
[f] Cook's D values obtained from SAS ver. 9.1.
[g] pK_a values were obtained from http://www.boomer.org/pkin/PK03/PK2003343.html.

phase. The pH of the mobile phase was adjusted using $^{s}_{w}$pH scale (Taillardat-Bertschinger et al., 2002) where glass electrode was calibrated using aqueous standard buffer solutions and the pH of mobile phase was adjusted after mixing buffer and acetonitrile, in order to obtain the desired pH. In addition to this, ionic strength of the mobile phase was corrected for dilution caused by the addition of acetonitrile in order to maintain a constant ionic strength of 0.01 M DPBS.

Retention times (t_R) and $\log k'_{IAM}$ values of amiloride, carbamazepine at pH 7.4 and ketorolac at pH 4 were obtained using 0.01 M DPBS as eluent on three separate days. Triplicate values were obtained for each compound on each day. The intra-day variations and inter-day variation of these data were determined for method validation.

2.4. Correlation analyses between $\log k'_{IAM}$ and human oral absorption

The HOA values for 28 drugs were collected from literature (Dollery, 1999; Zhao et al., 2001; Sugano et al., 2002) (Table 1). The SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA), and Prism software version 4 (Graph Pad Software Inc., San Diego, CA, USA) were used to compute the correlation and non-linear regression of $\log k'_{IAM}$ value with HOA. Besides verifying the assumption, model was checked for outliers using Cook's D statistics to identify influential observation.

3. Results

3.1. Validation of IAM chromatography method

Three compounds represented acid, base and neutral nature, i.e., ketorolac, amiloride and carbamazepine, were used for validating the IAM chromatographic method with respect to retention time reproducibility. These compounds were selected as they elute within a reasonable short period of time (<12 min) using 100% aqueous mobile phase condition. Intra-day and inter-day coefficient of variation (C.V.), as computed from the retention values (mean ± S.D.) depicted in Table 2, were <2 and 8% for all three compounds, respectively. Subsequently, the

Table 2
Intra- and inter-day variations of retention times (t_r) and inter-day variations of $\log k'_{IAM}$ values of amiloride, carbamazepine and ketorolac

Compounds ($n=3$)	t_r (min) [intra-day (mean ± S.D.)]	t_r (min) [inter-day (mean ± S.D.)]	$\log k'_{IAM}$ [inter-day (mean ± S.D.)]
Amiloride	2.46 ± 0.028	2.19 ± 0.166	0.883 ± 0.047
Carbamazepine	6.09 ± 0.029	6.08 ± 0.379	1.359 ± 0.037
Ketorolac	8.28 ± 0.033	8.61 ± 0.445	1.451 ± 0.05

calculated $\log k'_{IAM}$ values exhibited an inter-day C.V. of <6%, indicating a good reproducibility and robustness of the method. It was observed that conditioning the HPLC system initially with water for 10 min at a flow rate of 0.5 ml/min followed by mobile phase and proper cleaning of the column as per the recommendation from the manufacturer after experiments were essential in generating reproducible chromatographic profiles.

3.2. Determination of IAM capacity factor

According to pH partition theory, permeability of weak electrolytes is affected by the pH condition, following the change in compound dissociation (Hogben et al., 1959). In present study, we attempted to investigate the effect of mobile phase pH on the IAM capacity factor (k'_{IAM}) and its relationship with HOA. k'_{IAM} was determined for structurally diverse drugs represents acidic, basic, neutral and amphoteric nature with various pharmacodynamic and pharmacokinetic properties particularly low to high absorption and varying degree of ionization at physiological pH 7.4 using reverse phase HPLC. Initially, the influence of the pH of the mobile phase on k'_{IAM} was studied by chromatographing structurally diverse drugs including propranolol, diltiazem, naproxen, furosemide and carbamazepine at four different pH 4.5, 5.5, 6.5 and 7.4. Profiles of k'_{IAM} versus pH for above-mentioned drugs are shown in Fig. 1. Drugs with a negative charge at pH 7.4 (furosemide and naproxen) showed a higher retention in the acidic pH condition and drugs with positive charge at pH 7.4 (diltiazem and propranolol) showed a higher retention in alkaline pH conditions. The neutral drug like carbamazepine showed almost same k'_{IAM} values at all pH. Fig. 1 also demonstrates that the k'_{IAM} of acids is not well assessed if IAM assay is only performed at pH 7.4.

The k'_{IAM} for remaining drugs were measured using 0.01 M DPBS (pH 4.5–7.4) as the mobile phase, except for highly lipophilic drugs (imipramine, diltiazem, chlorpromazine, verapamil, indomethacin, diclofenac), which required an organic modifier to reduce the retention time (>30 min). IAM capacity factor of these drugs were determined by extrapolating to zero acetonitrile percentage using linear regression. Excellent linearity was found over the whole eluent composition range when $\log k'_{IAM}$ plotted against acetonitrile (v/v, %) in mobile phase, the correlation, R^2 being >0.98 for all drugs, with exception of diclofenac ($R^2 = 0.97$). Selected drugs showed $\log k'_{IAM}$ values in a large interval ranging from −1.560 for ganciclovir to 3.218 for chlorpromazine. A typical IAM chromatogram is shown in Fig. 2 where curve 1 represents the reference analyte citric acid and curves 2–9 represents different drugs retarded to different extent on IAM column. The retention of each drug in overlaid chromatograms was at the pH of their highest retention.

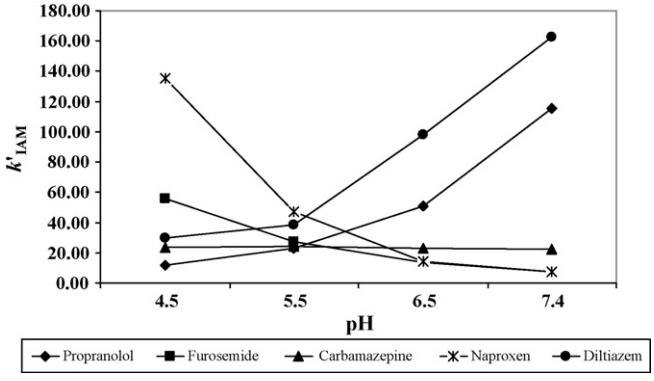

Fig. 1. pH dependent k'_{IAM} values for some compounds under investigation.

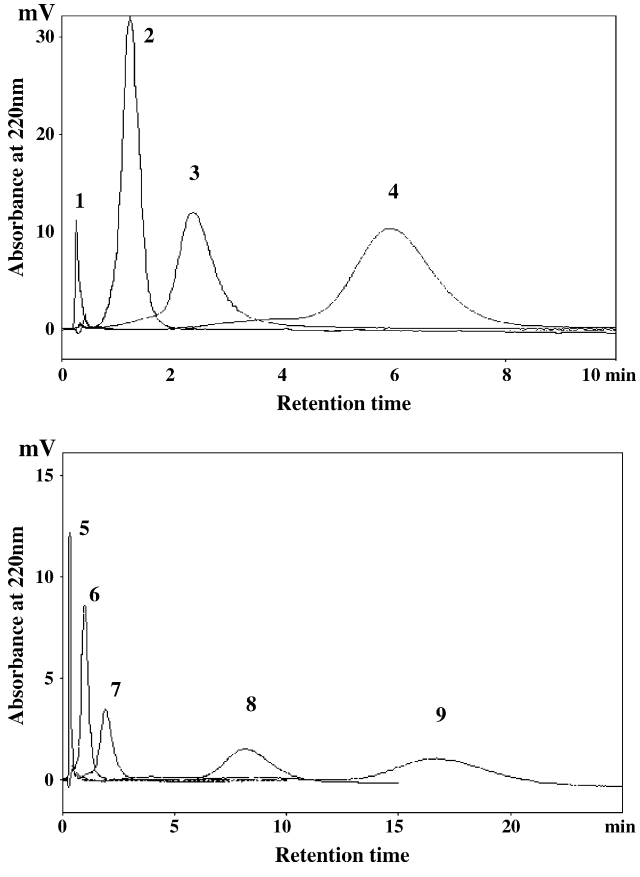

Fig. 2. Elution profiles of compounds on IAM column. Curves 1–9 corresponds to the elution profile of citric acid, hydrochlorothiazide, amiloride, carbamazepine, acyclovir, ranitidine, timolol, ketorolac and furosemide, respectively.

3.3. Relationship between human oral absorption and chromatographic parameter

Twenty-eight generic drugs with known human oral absorption were tested for their extent interaction with IAM stationary phase. Sigmoidal relationships between HOA and $\log k'_{IAM}$ were observed in present model, which is in agreement with previous observations (Stenberg et al., 2001; Sugano et al., 2001; Matsson et al., 2005). A non-linear regression curve fit showed that the training set $\log k'_{IAM}$ data from experimental model could be described by a sigmoidal function:

$$\%HOA = \frac{100}{(1 + (10^{\log k'_{IAM\,50\%}}/10^{\log k'_{IAM}})^{slope})} \quad (1)$$

where $\log k'_{IAM\,50\%}$ is the $\log k'_{IAM}$ at 50% HOA. A weak relationship ($R^2 = 0.7403$) was observed when $\log k'_{IAM}$ values at only pH 7.4 were taken into consideration, as shown in Fig. 3A. For a number of drugs, however, the $\log k'_{IAM}$ values measured at pH 7.4 leads to an underestimation of the fraction absorbed (ketorolac, naproxen, diclofenac and indomethacin). The $\log k'_{IAM}$ values for those compounds were up to 1 log unit lesser when measured at pH 7.4 rather than at pH other than 7.4. We observed high residual value (observed HOA minus predicted HOA) for these drugs.

Thus, to mimic an environment, which more closely resembles the conditions encountered as the substance moves through the GI-tract, capacity factor measurements were made at varying pH values, from pH 4.5–7.4, and the highest $\log k'_{IAM}$ value was taken into account for each compound to determine correlation. It can be seen from Fig. 3B that a reasonably good relationship ($R^2 = 0.8566$) is obtained between the two sets of data. There was substantial decrease in residual values for acidic drugs. This observation reveals that retention on IAM layer is strongly dependent on pH, especially compounds having pK_a value near the pH of the mobile phase used in determination of capacity factor. As seen in Fig. 3B, the $\log k'_{IAM}$ correlates to HOA closely through a trend line that consists of shallow slope region ($\log k'_{IAM}$: −1 to 2) and plateau region ($\log k'_{IAM}$: >2) where HOA levels off $\log k'_{IAM}$. The assumption for non-linear regression was verified by plotting a graph (not shown) of the residuals versus the values of the independent variable ($\log k'_{IAM}$). The residual values were randomly scattered about a reference line at 0 without any patterns or trends confirmed the validity of present model. The model was checked for the any influential observation, for which Cook's D statistics applied. Cook's D statistics is a measure of the simultaneous change in the parameter estimates when an observation is deleted from the analysis. In the present study, a suggested cutoff value for the influential observation is $D_i = 0.1429$. A suggested cutoff is an observation may have an adverse effect on the analysis if D_i is greater than $4/n$, where n is the sample size. The observed Cook's D value for each observation (Table 1, column 9) was less than the suggested cutoff. This result confirms that there is no influential observation in the model and the existence of a good correlation between the highest $\log k'_{IAM}$ values and HOA for a set of 28 drugs with diverse physicochemical characteristics.

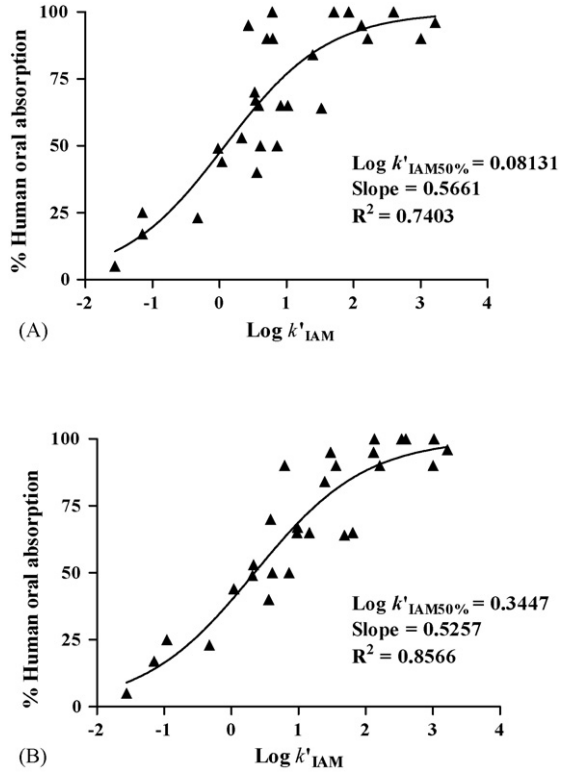

Fig. 3. Plot of % human oral absorption and $\log k'_{IAM}$ values for the set of compounds given in Table 1: (A) $\log k'_{IAM}$ values obtained at pH 7.4; (B) highest $\log k'_{IAM}$ values among pH 4.5–7.4.

4. Discussion

4.1. Determination of IAM capacity factor

It is generally believed that the apparent distribution constant of ionizable compounds is measured at the relevant pH rather than $\log k_{oct}$. And it should be correlated with membrane distribution and transport of drugs, because only the un-ionized form is supposed to be able to partition significantly into the lipid phase (Hansch et al., 1987). However, it was shown that the charged form of certain amines, but not of carboxylic acids, was able to partition into phospholipids vesicles along with the uncharged form (Austin et al., 1995). The GI-tract exhibits a considerable pH gradient, and pH partition hypothesis predicts that the absorption of ionizable drugs may be location specific. Absorption of drug products generally takes place in small intestine, in a pH range 4.5–8.0. This suggests that weak acids ought to be better absorbed in the jejunum and weak bases in the ileum (Avdeef, 2001). Therefore, it is necessary to utilize appropriate pH condition for the adequate prediction of oral absorption.

In present study, initially five drugs, propranolol, diltiazem, naproxen, furosemide and carbamazepine were studied at pH 4.5, 5.5, 6.5 and 7.4 to investigate the influence of the mobile phase pH on their k'_{IAM} value. The results indicated that positively and negatively charged drugs showed higher retention at alkaline and acidic pH condition, respectively. Neutral drug was

not affected by pH change of mobile phase and showed almost same k'_{IAM} values at all pH studied (Fig. 1). This pH dependence of k'_{IAM} was in accordance with pH partition theory. This can be explained by probable changes in conformation and/or charge distribution occurring in phospholipids as the pH of the eluent approaches their $pK_a \sim 2.0$ (Pauletti and Wunderli-Allenspach, 1994).

Highly lipophilic drugs like imipramine, diltiazem, chlorpromazine, verapamil, indomethacin and diclofenac did not elute within a reasonable time (~30 min) with 100% aqueous mobile phase and therefore the addition of acetonitrile to the eluent was needed. The percentage of acetonitrile used was not more than 30% since this would disrupt the water structure (Morse and Pidgeon, 2001). The pH and ionic strength of the mobile phases were adjusted in the presence of the acetonitrile (5–30%, v/v) to minimize the deviation in the $\log k'_{IAM}$. Up to 40% deviation of the $\log k'_{IAM}$ value was reported when the pH of acetonitrile containing mobile phases were not readjusted to initial values (Caldwell et al., 1998). The $\log k'_{IAM}$ values for these drugs obtained by extrapolating linear regression line to zero acetonitrile percentage. The $\log k'_{IAM}$ of propranolol measured at 100% aqueous mobile phase was 2.119, which was close to the $\log k'_{IAM}$ value determined by extrapolation method 2.051, confirmed the reliability of extrapolation method.

The $\log k'_{IAM}$ values obtained were in range of −1.560 (ganciclovir) to 3.218 (chlorpromazine). This result reflects the disparate partition behavior of the selected drugs and their diverse phospholipids membrane affinities (Table 1; Fig. 2). We note that such elution profiles of the drugs have not been demonstrated with IAM chromatography.

4.2. Relationship between human oral absorption and chromatographic parameter k'_{IAM}

From a physiological point of view, absorption can be regarded to take place at mucosal membrane of the gastrointestinal (GI) tract. Once drug molecule enters the membrane, they can be deemed absorbed from the GI lumen (Chiou, 2001). Most of the absorption data from the literature are based on one of the three main methods outline in literature, i.e., bioavailability, excretion in urine and feces following oral administration and the ratio of cumulative urinary excretion of drug related material following oral and intravenous administration (Zhao et al., 2001). The artificial membrane permeability assay is a method for the assessment of passive transcellular permeation. Compounds smaller than MW 200 are absorbed via paracellular pathway (Liu et al., 2002; Sugano et al., 2001; Wohnsland and Faller, 2001); therefore they were excluded from data set. In addition, compounds absorbed via the active transport pathway were also excluded (Bretschneider et al., 1999). We selected compounds whose %HOA was ≤90 because prediction of this range of oral absorption is anticipated for the in vitro method in the discovery process. The molecular weight and HOA values of selected compounds are shown in Table 1.

We compared the measured $\log k'_{IAM}$ with the human oral absorption data of 28 drugs in the literature. In Fig. 3B, the highest $\log k'_{IAM}$ value among pH 4.5–7.4 showed good correlation ($R^2 = 0.8566$) with HOA. Similar findings were also observed in the case of PAMPA and Caco-2 cells. Artificial membrane permeability measurements have exhibited best correlation with in vivo intestinal absorption (Wohnsland and Faller, 2001; Zhu et al., 2002) and Caco-2 permeability (Kerns et al., 2004) at the pH of highest permeability. Recently, pH dependent passive and active transport of acidic drugs across Caco-2 cells was investigated (Neuhoff et al., 2005). They also observed the influence of the pH conditions on transport of truly passively transported acidic drugs across Caco-2 cell monolayer in a way that could be misinterpreted as active transport. Present studies further suggest that it is useful to measure k'_{IAM} at both an acidic pH, as well as at the more traditional pH 7.4.

When 13 compounds (compound no. 1–3, 7, 12, 13, 15, 17, 24–28; Table 1) mainly basic and neutral (at pH 7.4) in nature with higher retention at pH 7.4 treated separately from the other drugs, their capacity factors showed good correlation with HOA ($R^2 = 0.8858$). Basic compounds ($pK_a > 8$) which remain almost ionized (80 to ~100%) at pH 7.4 showed lipophilicity based retention regardless of degree of ionization. This observation is in agreement with a previous study suggesting that ionized basic drugs strongly interact with the IAM stationary phase and their retention becomes as strong as if they were uncharged (Barbato et al., 1997). Twelve compounds (compound no. 6, 8, 9, 11, 14, 16, 18–23; Table 1) mainly acidic and amphoteric in nature showed less $\log k'_{IAM}$ values at more traditional pH 7.4 compare to the other pH (e.g., pH 4.5), result into underestimation of their oral absorption particularly compounds with pK_a value ≤4.5. Thus, they clearly form a subgroup that behaves differently in IAM chromatography at acidic pH with improvement in overall relationship. The absorption profile of acidic drugs in the GI-tract can in general be explained by the pH-partition hypothesis (Shore et al., 1957), which states that a lipid membrane allows the passage of uncharged but not charged drug species. Additionally, the acidic microclimate at the intestinal mucosal surface would increase the amount of uncharged weakly acidic drug available for passive diffusion (Daniel et al., 1985; McEwan and Lucas, 1990).

Interestingly, all the compounds in the data set lay with the sigmoidal relationship despite the fact several of them are at least partly transported by various active transporter and paracellular route. Previous in situ study indicated that the intestinal absorption of lisinopril is a saturable carrier-mediated process via the dipeptide transporter system (Friedman and Amidon, 1989). Although the IAM capacity factor of this drug correlated well with HOA. Even if transporter proteins carry them, contribution by it to total cell permeation could be small. The higher inhibition constant compare to other ACE-inhibitors and 3D structural analysis of lisinopril using molecular modeling techniques reveals that intramolecular hydrogen bond formation is responsible for decreased carrier affinity (Swaan et al., 1995). This was evaluated based on its ability to inhibit the transport of cephalexin. Atenolol, ranitidine and hydrochlorothiazide (Collett et al., 1996), famotidine (Lee et al., 2002) and furosemide (Flanagan et al., 2002) have been reported to be absorbed via a paracellular pathway. But for these compounds most of the in vitro models particularly artificial membranes,

i.e., PAMPA, immobilized liposomes and IAM chromatography showed good correlation with Caco-2 cells permeability (Kerns et al., 2004) and human intestinal absorption (Sugano et al., 2001; Zhu et al., 2002; Karlsson et al., 2005; Yen et al., 2005). It was also reported that compounds with molecular weight over 200 are absorbed only to minor extent by the paracellular route (Fujikawa et al., 2005). This result suggests the importance of absorption by a transcellular mechanism for the drugs.

4.3. Other IAM chromatography studies

Some data concerning the relation of IAM capacity factor with drug permeation and pharmacokinetic behavior were published earlier. The relationship between $\log k'_{IAM}$ at pH 7 and permeability across Caco-2 cells of six β-blockers showed hyperbolic relationship (Osterberg et al., 2001). The correlation between $\log k'_{IAM}$ retention at pH 7.4 with Caco-2 uptake for nine HIV protease inhibitors improved (R^2 improving from 0.39 to 0.91) when molecular weight (MW) and hydrogen bonding capacity were included in the regression (Stewart et al., 1998). Good correlation was demonstrated ($R = 0.941$) between $\log k'_{IAM}$ values at pH 7.4 and oral absorption in mice for set of structurally related 11 cephalosporin analogs (Pidgeon et al., 1995). However, the investigated sets comprised only of structurally similar drugs, thus seriously restricting any extrapolation and forbidding the calculation of any correlation except linear ones.

Some authors examined correlation between IAM capacity factor and gastrointestinal absorption, Caco-2 cells uptake using diverse set of compounds. The k'_{IAM} of structurally diverse compounds at pH 5.4 and 7.4 coupled with MW (k'_{IAM}/MW) showed good correlation with rat small intestine absorption ($R = 0.858$) and Caco-2 cells ($R = 0.854$) permeability coefficient (Pidgeon et al., 1995). The k'_{IAM} at pH 7 coupled with molar volume showed good correlation (R^2 improving from 0.77 to 0.83) with passive permeability through rat everted gut sacs (Genty et al., 2001). Recent study showed poor correlation ($R^2 = 0.227$) between $\log k'_{IAM}$ value at pH 7 and Caco-2 absorption values. However, an optimal correlation ($R^2 = 0.840$) was obtained after inclusion of molecular size factor in regression analysis and also by excluding the 11 compounds having associated factors, which were affecting their biopartitioning (Chan et al., 2005). Most recent study reported that human intestinal absorption was reciprocally correlated to the negative value of the capacity factor determined at pH 5.4 ($R = 0.64$). The correlation further improved ($R = 0.83$) with addition of molecular descriptor representing molecular size, shape, solubility and polarity (Yen et al., 2005). In most of the above experiments, the k'_{IAM} measurements have been performed at single pH, which appears not to be a typical pH of small intestine; leads to underestimation of the oral absorption of some positively charged and particularly negatively charged drugs. The correlation ($R^2 = 0.8566$) between $\log k'_{IAM}$ (highest value among pH 4.5–7.4) and human oral absorption with present experimental condition is comparable and in some cases better than already conducted intestinal permeability experiments with IAM chromatography without incorporating physicochemical descriptors.

5. Conclusion

The relative importance of each mechanism to total permeation is likely to differ with the characteristics of the specific barrier (e.g., gastrointestinal, BBB, cell type). Such an approach could provide specific guidance for property-based design. The present work demonstrates that IAM chromatography can be used to predict the intestinal passive transcellular absorption of structurally diverse drugs. The data generated with our training set emphasize the importance of chromatographic condition particularly effect of ionization at given pH during determination of IAM capacity factor for better correlation with GI-tract absorption of ionizable drugs. Good correlation between IAM capacity factor and HOA data for a compound indicates a predominance of passive diffusion particularly transcellular in its permeation. This technique is not sensitive to active uptake or efflux mechanisms, but this is also the case for other permeability assays such as PAMPA and immobilized liposome, and is not a problem at an early screening stage in the drug discovery process. Hence it is not a replacement for Caco-2 cell monolayer, but rather a complementary method for the preselection of compounds, which at a later stage can be tested for their biochemical properties. Finally, the training set includes compound with MW < 600, which is important when considering GI-tract absorption because substances with molecular weights lower than 200 might cross the GI-tract barrier via paracellular route while for higher molecular weight compounds (MW > 600), molecular size becomes rate limiting. For the later compounds one may combine the IAM indices with molecular size related descriptor like MW.

In conclusion, IAM chromatography provides fast and simple tool to quantify drug–membrane interactions separately from other relevant factors such as molecular weight or hydrogen bonding capacity. This may help in designing of new chemical entities with appropriate oral absorption and for the selection of candidates in the early stage of drug discovery process.

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References

Abraham, M.H., Takacs-Novak, K., Mitchell, R.C., 1997. On the partition of ampholytes: application to blood–brain distribution. J. Pharm. Sci. 86, 310–315.

Artursson, P., Karlsson, J., 1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem. Biophys. Res. Commun. 175, 880–885.

Austin, R.P., Davis, A.M., Manners, C.N., 1995. Partitioning of ionizing molecules between aqueous buffers and phospholipid vesicles. J. Pharm. Sci. 84, 1180–1183.

Avdeef, A., Barrett, D.A., Shaw, P.N., Knaggs, R.D., Davis, S.S., 1996. Octanol-, chloroform-, and propylene glycol dipelargonat-water partitioning of morphine-6-glucuronide and other related opiates. J. Med. Chem. 39, 4377–4381.

Avdeef, A., 2001. Physicochemical profiling (solubility, permeability and charge state). Curr. Top. Med. Chem. 1, 277–351.

Barbato, F., La Rotonda, M.I., Quaglia, F., 1997. Chromatographic indexes on immobilized artificial membranes for local anesthetics: relationships with activity data on closed sodium channels. Pharm. Res. 14, 1699–1705.

Beigi, F., Gottschcalk, I., Hagglund, C.L., Haneskog, L., Brekkan, E., Zhang, Y., Osterberg, T., Lundahl, P., 1998. Immobilized liposome and biomembrane partitioning chromatography of drugs for prediction of drug transport. Int. J. Pharm. 164, 129–137.

Bretschneider, B., Brandsch, M., Neubert, R., 1999. Intestinal transport of beta-lactam antibiotics: analysis of the affinity at the H+/peptide symporter (PEPT1), the uptake into Caco-2 cell monolayers and the transepithelial flux. Pharm. Res. 16, 55–61.

Caldwell, G.W., Masucci, J.A., Evangelisto, M., White, R., 1998. Evaluation of the immobilized artificial membrane phosphotidylcholine: drug discovery column for high-performance liquid chromatographic screening of drug–membrane interactions. J. Chromatogr. A 800, 161–169.

Camenisch, G., Folkers, G., van de Waterbeemd, H., 1996. Review of theoretical passive absorption models: historical background, recent developments and limitations. Pharm. Acta Helv. 71, 309–327.

Chan, E.C.Y., Tan, W.L., Ho, P.C., Fang, L.J., 2005. Modeling Caco-2 permeability of drugs using immobilized artificial membrane chromatography and physicochemical descriptors. J. Chromatogr. A 1072, 159–168.

Chiou, W.L., 2001. The rate and extent of oral bioavailability versus the rate and extent of oral absorption: clarification and recommendation of terminology. J. Pharmacokinet. Pharmacodyn. 28, 3–6.

Collett, A., Sims, E., Walker, D., He, Y.L., Ayrton, J., Rowland, M., Warhurst, G., 1996. Comparison of HT29-18-C1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption. Pharm. Res. 13, 216–221.

Comer, J., Tam, K., 2001. Lipophilicity profiles: theory and measurement. In: Testa, B., van de Waterbeemd, H., Folkers, G., Guy, R.H. (Eds.), Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies. Wiley-VHCA, Zurich, pp. 275–304.

Conradi, R.A., Burton, P.S., Borchardt, R.T., 1996. Physico-chemical and biological factors that influence a drug's cellular permeability by passive diffusion. In: Pliska, V., Testa, B., van de Waterbeemd, H. (Eds.), Lipophilicity in Drug Action and Toxicology. VCH Publishers, Germany, pp. 233–252.

Daniel, H., Neugebauer, B., Kratz, A., Rehner, G., 1985. Localization of acid microclimate along intestinal villi of rat jejunum. Am. J. Physiol. 248, G293–G298.

Dollery, C.T., 1999. Therapeutic Drugs, 2nd ed. Churchill Livingstone, Edinburgh.

Flanagan, S.D., Takahashi, L.H., Liu, X., Benet, L.Z., 2002. Contributions of saturable active secretion, passive transcellular, and paracellular diffusion to the overall transport of furosemide across adenocarcinoma (Caco-2) cells. J. Pharm. Sci. 91, 1169–1177.

Friedman, D.I., Amidon, G.L., 1989. Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29,852. J. Pharm. Sci. 78, 995–998.

Fujikawa, M., Ano, R., Nakao, K., Shimizu, R., Akamatsu, M., 2005. Relationships between structure and high-throughput screening permeability of diverse drugs with artificial membranes: application to prediction of Caco-2 cell permeability. Bioorg. Med. Chem. 13, 4721–4732.

Genty, M., Gonzalez, G., Clere, C., Desangle-Gouty, V., Legendre, J.Y., 2001. Determination of passive absorption through the rat intestine using chromatographic indices and molar volume. Eur. J. Pharm. Sci. 12, 223–229.

Hansch, C., Bjorkroth, J.P., Leo, A., 1987. Hydrophobicity and central nervous system agents: on the principle of minimal hydrophobicity in drug design. J. Pharm. Sci. 76, 663–687.

Hogben, C.A.M., Tocco, D.J., Brodie, B.B., Schanker, L.S., 1959. On the mechanism of intestinal absorption of drugs. J. Pharmacol. Exp. Ther. 125, 275–282.

Kansy, M., Senner, F., Gubernator, K., 1998. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. J. Med. Chem. 41, 1007–1010.

Karlsson, A., Widegren, H., Green, C.E., Hamalainen, M.D., Westerlund, L., Karlsson, R., Fenner, K., van de Waterbeemd, H., 2005. Biosensor analysis of the interaction between drug compounds and liposomes of different properties: a two-dimensional characterization tool for estimation of membrane absorption. J. Pharm. Sci. 94, 25–37.

Kerns, E.H., Di, L., Petusky, S., Farries, M., Ley, R., Jupp, P., 2004. Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assays in drug discovery. J. Pharm. Sci. 93, 1440–1453.

Lee, K., Ng, C., Brouwer, K.L., Thakker, D.R., 2002. Secretory transport of ranitidine and famotidine across Caco-2 cell monolayers. J. Pharmacol. Exp. Ther. 303, 574–580.

Liu, H., Ong, S., Glunz, L., Pidgeon, C., 1995. Predicting drug–membrane interactions by HPLC: structural requirements of chromatographic surfaces. Anal. Chem. 67, 3550–3557.

Liu, X.Y., Nakamura, C., Yang, Q., Kamo, N., Miyake, J., 2002. Immobilized liposome chromatography to study drug–membrane interactions. Correlation with drug absorption in humans. J. Chromatogr. A 961, 113–118.

Matsson, P., Bergstrom, C.A.S., Nagahara, N., Tavelin, S., Norinder, U., Artursson, P., 2005. Exploring the role of different drug transport routes in permeability screening. J. Med. Chem. 48, 604–613.

McEwan, G.T., Lucas, M.L., 1990. The effect of *E. coli* STa enterotoxin on the absorption of weakly dissociable drugs from rat proximal jejunum in vivo. Br. J. Pharmacol. 101, 937–943.

Morse, K.L., Pidgeon, C., 2001. Importance of the mobile phase in immobilized artificial membrane chromatography. In: Testa, B., van de Waterbeemd, H., Folkers, G., Guy, R. (Eds.), Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies. Wiley-VHCA, Zurich, pp. 429–445.

Neuhoff, S., Ungell, A.L., Zamora, I., Artursson, P., 2005. pH-Dependent passive and active transport of acidic drugs across Caco-2 cell monolayers. Eur. J. Pharm. Sci. 25, 211–220.

Osterberg, T., Svensson, M., Lundahl, P., 2001. Chromatographic retention of drug molecules on immobilized liposomes prepared from egg phospholipids and from chemically pure phospholipids. Eur. J. Pharm. Sci. 12, 427–439.

Pagliara, A., Testa, B., Carrupt, P.A., Jolliet, P., Morin, C., Morin, D., Urien, S., Tillement, J.P., Rihoux, J.P., 1998. Molecular properties and pharmacokinetic behavior of cetirizine, a zwitterionic H1-receptor antagonist. J. Med. Chem. 41, 853–863.

Pauletti, G.M., Wunderli-Allenspach, H., 1994. Partition coefficients in vitro: artificial membranes as a standardized distribution model. Eur. J. Pharm. Sci. 1, 273–282.

Pidgeon, C., Ong, S., Liu, H., Qiu, X., Pidgeon, M., Dantzig, A.H., Munroe, J., Hornback, W.J., Kasher, J.S., Glunz, L., Szczerba, T., 1995. IAM chromatography: an in vitro screen for predicting drug membrane permeability. J. Med. Chem. 38, 590–594.

Prentis, R.A., Lis, Y., Walker, S.R., 1988. Pharmaceutical innovation by the seven UK-owned pharmaceutical companies (1964–1985). Br. J. Clin. Pharmacol. 25, 387–396.

Shore, P.A., Brodie, B.B., Hogben, C.A., 1957. The gastric secretion of drugs: a pH partition hypothesis. J. Pharmacol. Exp. Ther. 119, 361–369.

Stenberg, P., Norinder, U., Luthman, K., Artursson, P., 2001. Experimental and computational screening models for the prediction of intestinal drug absorption. J. Med. Chem. 44, 1927–1937.

Stewart, B.H., Chung, F.Y., Tait, B., Blankley, C.J., Chan, O.H., 1998. Hydrophobicity of HIV protease inhibitors by immobilized artificial membrane chromatography: application and significance to drug transport. Pharm. Res. 15, 1401–1406.

Sugano, K., Hamada, H., Machida, M., Ushio, H., Saitoh, K., Terada, K., 2001. Optimized conditions of bio-mimetic artificial membrane permeation assay. Int. J. Pharm. 228, 181–188.

Sugano, K., Takata, N., Machida, M., Saitoh, K., Terada, K., 2002. Prediction of passive intestinal absorption using bio-mimetic artificial membrane per-

meation assay and the paracellular pathway model. Int. J. Pharm. 241, 241–251.

Swaan, P.W., Stehouwer, M.D., Tukker, J.J., 1995. Molecular mechanism for the relative binding affinity to the intestinal peptide carrier. Comparison of three ACE-inhibitors: enalapril, enalaprilat, and lisinopril. Biochim. Biophys. Acta 1236, 31–38.

Taillardat-Bertschinger, A., Carrupt, P.A., Barbato, F., Testa, B., 2003. Immobilized artificial membrane HPLC in drug research. J. Med. Chem. 46, 655–665.

Taillardat-Bertschinger, A., Galland, A., Carrupt, P.A., Testa, B., 2002. Immobilized artificial membrane liquid chromatography: proposed guidelines for technical optimization of retention measurements. J. Chromatogr. A 953, 39–53.

Testa, B., Carrupt, P.A., Gaillard, P., Tsai, R.S., 1996. Intramolecular interactions encoded in lipophilicity: their nature and significance. In: Pliska, V., Testa, B., van de Waterbeemd, H. (Eds.), Lipophilicity in Drug Action and Toxicology. VCH Publishers, Germany, pp. 49–71.

van de Waterbeemd, H., Testa, B., 1987. The parameterization of lipophilicity and other structural properties in drug design. In: Testa, B. (Ed.), Advances in Drug Research, vol. 16. Academic Press, London, pp. 87–227.

Wohnsland, F., Faller, B., 2001. High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes. J. Med. Chem. 44, 923–930.

Williams, D.A., Lemke, T.L., 2002. Foye's Principles of Medicinal Chemistry, 5th ed. Lippincott Williams and Wilkins, Baltimore.

Yang, C.Y., Cai, S.J., Liu, H., Pidgeon, C., 1996. Immobilized artificial membranes—screens for drug–membrane interactions. Adv. Drug Deliv. Rev. 23, 229–256.

Yen, T.E., Agatonovic-Kustrin, S., Evans, A.M., Nation, R.L., Ryand, J., 2005. Prediction of drug absorption based on immobilized artificial membrane (IAM) chromatography separation and calculated molecular descriptors. J. Pharm. Biomed. Anal. 38, 472–478.

Zhao, Y.H., Le, J., Abraham, M.H., Hersey, A., Eddershaw, P.J., Luscombe, C.N., Butina, D., Beck, G., Sherborne, B., Cooper, I., Platts, J.A., 2001. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure–activity relationship (QSAR) with the Abraham descriptors. J. Pharm. Sci. 90, 749–784.

Zhu, C., Jiang, L., Chen, T.M., Hwang, K.K., 2002. A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential. Eur. J. Med. Chem. 37, 399–407.