International Journal of Pharmaceutics 360 (2008) 96–106

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Prediction of oral absorption in humans by experimental immobilized artificial membrane chromatography indices and physicochemical descriptors

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ARTICLE INFO

Article history:
Received 28 September 2007
Received in revised form 14 April 2008
Accepted 15 April 2008
Available online 22 April 2008

Keywords:
Immobilized artificial membrane (IAM)
Partition coefficients
Physicochemical descriptors
Non-linear regression
Oral absorption

ABSTRACT

The purpose of the present study was to examine the human oral absorption (HOA) predictability of the experimentally determined immobilized artificial membrane (IAM) chromatography capacity factor ($\log k'_{IAM}$) in conjunction with physicochemical descriptors. Transcellular permeation was modeled based on determination of $\log k'_{IAM}$ considering pH partition hypothesis, and the independent variables were polar surface area (PSA) and molecular weight (MW). The correlation between $\log k'_{IAM}$ determined at different pH and *n*-octanol/water partition coefficient ($\log P$) and contribution of polarity (PSA) and size (MW) in the transcellular permeation model were the extension to the previous work. A data set of 37 compounds with partition coefficient values taken from the literature was employed to show importance of ionic interaction in oral absorption prediction. The highest $\log k'_{IAM}$ value among screened pH 4.5, 5.5, 6.5 and 7.4 ($\log k'_{IAM}{}^{4.5-7.4}$) in conjunction with PSA predicted HOA with coefficient of determination (CD) of 0.9001 compare to $\log k'_{IAM}{}^{4.5-7.4}$ alone with CD of 0.8454 after excluding bretylium from the set of 28 structurally diverse drugs for known reason. PSA helped to avoid over estimation of HOA for amiloride, famotidine and furosemide. The model was tested for its applicability in drug development program and found to predict oral absorption using physically meaningful and structurally related properties making them relatively straightforward for a medicinal chemist to interpret.

1. Introduction

The ideal situation for the medicinal chemist is that the human metabolism and pharmacokinetics of a compound can be predicted on its physicochemical properties and molecular structure. The prediction of oral bioavailability is very challenging due to the fact that bioavailability is a complex function of many biological and physicochemical factors, such as dissolution in the gastrointestinal tract, intestinal membrane permeation, intestinal and hepatic first-pass metabolism, and even the dosage form. Among absorption, distribution, metabolism and excretion (ADME) properties, good oral absorption is one of the most desirable attributes of a new drug. On current stage, major efforts are focused on the prediction of human oral absorption (HOA), because the first step for obtaining high oral bioavailability is to achieve good oral absorption. Absorption is determined by solubility, permeability (active and passive), and its first-pass metabolism across the gut wall. The passive transcellular permeability of a compound is often modeled on lipophilicity, molecular size, and/or hydrogen bonding. Analysis of the structures of orally administered drugs, and of drug candidates, as pioneered by Lipinski et al. (1997), has so far been the primary guide to correlate physical properties with successful drug development. Since then numerous classification and regression models for the prediction of HOA were reported by applying a variety of statistical and machine-leaning approaches, which include multiple linear regression (Zhao et al., 2001), non-linear regression (Palm et al., 1997), partial least squares regression (Osterberg and Norinder, 2000), classification and regression trees (Deconinck et al., 2005), artificial neural networks (Agatonovic-Kustrin et al., 2001) and so forth.

The literature reports linear, bilinear, sigmoidal, or parabolic relationship between permeability and lipophilicity (Kubinyi, 1993). Good correlation between lipophilicity and permeability can often be obtained within a co-generic series. Lipophilicity is usually measure as partition coefficient, *P*, between the two immiscible phases and is often expressed as $\log P$. For ionizable compounds, distribution coefficient ($\log D$) is actually the more interesting parameter; since it gives the lipophilicity at a relevant pH. It is generally assumed that only the unionized species can partition into the lipid phase (Leo et al., 1969). Although ionized species are

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doi:10.1016/j.ijpharm.2008.04.025

probably able to partition into the lipid phase, the partition coefficient of such an ionized species is usually about three orders of magnitude lower than the partition coefficient of neutral species (Avdeef, 1996). This indicates that selected in vitro model for prediction of oral drug absorption must take ionization into account.

The lipophilicity by itself is inadequate for the estimation of the solute's ability to penetrate a membrane barrier. Therefore, both hydrophobic effects and hydrogen bonding forces must be considered rather than just lipophilicity (Conradi et al., 1996). To estimate H-bonding ability, models use polar surface area (PSA) and it has received a great deal of attention as potential predictors of the membrane transport. This parameter is easy to understand and most importantly, provides good correlation with experimental transport data (Palm et al., 1996, 1998; Krarup et al., 1998; Winiwarter et al., 1998; Kelder et al., 1999; Stenberg et al., 1999a,b; Ertl et al., 2000). The PSA has demonstrated a non-linear relationship between PSA and permeability, with permeability declining sigmoidally as PSA increases. There are reports in the literature showing correlation between transcellular and/or paracellular permeability and molecular size (Shah et al., 1989; van de Waterbeemd et al., 1996). Molecular size also determines the absorption route; a drug can take (Artursson et al., 1993). A simple molecular size descriptor, the molecular weight (MW) is often used in absorption modeling (Yoon et al., 2004; Chan et al., 2005).

Immobilized artificial membrane (IAM) consists of ordered phospholipid surface that supports drug membrane partitioning and ionic interactions (Pidgeon et al., 1995). They are successfully employed for the determination of partition coefficient of ionized and unionized compounds. Considering this fact, in previous paper we reported a new relationship between HOA and highest $\log k'_{IAM}$ among pH 4.5–7.4 ($\log k'_{IAM}{}^{4.5-7.4}$) (Kotecha et al., 2007). It was shown that $\log k'_{IAM}$ determined at different pH had better predictive performance ($R^2 = 0.8566$) than only at pH 7.4 ($R^2 = 0.7403$).

Therefore in this paper we aimed to study advantages of $\log k'_{IAM}{}^{4.5-7.4}$ compare to *n*-octanol/water partition coefficients to explain lipophilicity and charge state, which contributes largely into lipophilic interaction of drugs with phospholipids membrane. Further to improve the physiological basis of in vitro model for oral absorption prediction, physicochemical descriptors are included. For a set of 28 drugs consisting of neutral, acidic, basic and amphoteric compounds, covers a fair range of MW and PSA. $\log k'_{IAM}{}^{4.5-7.4}$ values were determined using the established IAM chromatographic technique and were correlated with physicochemical descriptors using non-linear multiple regression analysis. The test set was evaluated for their oral absorption property using developed regression model. The findings of the study and their implications are elaborated in the subsequent sections.

2. Materials and methods

2.1. Chemicals

All compounds investigated in this study as mentioned in Tables 1 and 2 were all supplied by Torrent Pharmaceutical Ltd. (Ahmedabad, India) and used without further purification. The analytical grade solvents methanol and acetonitrile were purchased from Ranbaxy (New Delhi, India). The HPLC aqueous mobile phase was Dulbecco's phosphate buffer saline (DPBS) purchased from HIMEDIA (Mumbai, India) and dissolved in deionized water obtained from Milli-Q water purification system from Millipore (Banglore, India). The orthophosphoric acid for the pH adjustment and citric acid were obtained from Merck (Mumbai, India).

2.2. Determination of capacity factors at different pH by IAM-HPLC

The retention measurements were performed by HPLC which 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). Data acquisition and processing were performed on LC-10 software. The HPLC column was an IAM.PC.DD2 column (1 cm × 3 mm, 12 μm, 300 A°) which was purchased from Regis Technology (Morton Grove, IL, USA).

The eluents were mixture of acetonitrile (5–30% v/v) and phosphate buffer saline at pH 4.5, 5.5, 6.5 and 7.4 (4.5–7.4). 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. 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. The flow rate was 0.5 ml/min. The mobile phases were filtered through 0.45 μm filters from Millipore. The column temperature was maintained at 25 °C using column oven CTO-10A (Shimadzu, Kyoto, Japan). Stock solutions (0.1 mg/ml) of compounds were prepared in either water or methanol. They were further diluted suitably before analysis with DPBS and the dilution factor was between 5 and 10 times. The injection volume was 5 μl. UV detection was monitored at 220 and 254 nm. The HPLC system was conditioned by passing the water for 10 min at a flow rate of 0.5 ml/min followed by mobile phase till a stable UV base line observed before injecting the samples.

Chromatographic retention data are mean of at least three determinations and are expressed by the logarithm of capacity factor, $\log k'_{IAM}$; defined as $\log k'_{IAM} = \log[(t_r - t_0)/t_0]$ where t_r and t_0 are the retention times of the solute and the non-retained compound (citric acid), respectively. For elution of more lipophilic compounds, mixture of acetonitrile/phosphate buffer (pH 4.5–7.4) containing up to 30% organic modifier were used. The $\log k'_{IAM}$ values referring to the buffer only mobile phase were extrapolated by plotting the $\log k'_{IAM}$ values and the applied acetonitrile concentration. The intercept of the straight line was used as the extrapolated $\log k'_{IAM}$ to buffer only mobile phase.

2.3. Data set and statistical analysis

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 (Sugano et al., 2001; Wohnsland and Faller, 2001; Liu et al., 2002) therefore they were excluded from data set. In addition, compounds absorbed via the active transport pathway were also excluded (Bretschneider et al., 1999). The %HOA values for 38 drugs under investigation (Table 1) were collected from literature (Dollery, 1999; Zhao et al., 2001; Sugano et al., 2002). Additional data set was considered to study the molecular factors influencing retention on IAM (Table 2). Above-mentioned compound selection criteria were not applicable to Table 2 compounds other than mentioned from Table 1. MW and PSA values were obtained from literature (Veber et al., 2002) and for some of the compounds values obtained from Cerius^2 ADME module v4.10. (Accelrys, USA). *C* log *P* were calculated using the *C* log *P* v4.0 (BioByte Corp., USA). As it has been demonstrated using an independent data set, *C* log *P* is more accurate predictor of the octanol/water partition (Machatha and Yalkowsky, 2005), we considered calculated values as experimental log *P* values in text. Experimental *n*-octanol/water partition coefficient values

at pH 7.4 were taken from literature (Kansy et al., 2001; Zhu et al., 2002). Ionization constant (HA and HB+) values were collected from literatures (Sugano et al., 2002; Williams and Lemke, 2002). All values have been mentioned against each compound under respective heading (Tables 1 and 2). For linear and non-linear regression analysis, a statistical package Kyplot v2.0 (KyensLab Inc., Tokyo, Japan) was used on a personal computer. Following descriptive information is provided for linear as well as non-linear regression analysis: number of observations used in the analysis (n), coefficient of determination (CD) (R^2), and standard error (S.E.) of the estimate(s).

3. Results

3.1. Determination of IAM capacity factors

The set of 52 structurally diverse compounds considered to study retention characteristics on IAM column and to build model for HOA prediction includes basic, acidic, neutral and amphoteric compounds was determined in same manner as described previously (Kotecha et al., 2007). Only seven compounds (chlorpromazine, diclofenac, diltiazem, imipramine, indomethacin, nimodepine and verapamil) required an organic modifier to reduce

Table 1
Physicochemical properties, observed and predicted %HOA

No.	Compound	MW[a]	pK_a[b]		log P[c]	log $D_{oct}^{7.4}$[d]	log k'_{IAM} (pH 4.5–7.4)[e]	PSA[f] (Å^2)	Observed %HOA[g]	Predicted %HOA[h]
			HA	HB+						
Training set										
1	Acyclovir	225	9.3	2.3	−2.42	−1.86	−1.150	119.1	17 (13–21)	19
2	Amiloride	230		8.7	−0.55	−0.86	0.862	156.8	50	50
3	Atenolol	266		9.6	−0.11	−1.29	0.039	84.6	44	51
4	Bretylium	244			−1.25		−0.326	13.0	23	–
5	Carbamazepine	236			1.98	1.78	1.392	46.3	84 (73–96)	85
6	Chlorothiazide[j]	295	6.8, 9.5		−0.24	−0.05	0.316	119.9	49(36–61)	47
7	Chlorpromazine	319		9.3	5.80	3.38	3.218	6.5	96	98
8	Chlorthalidone	338	9.4		0.45	0.78	1.161	109.5	65 (60–70)	69
9	Ciprofloxacin	331	6	8.8	−1.15		0.984	74.6	67	74
10	Dexamethasone	393			1.78	1.83	1.560	94.8	90	79
11	Diclofenac	296	4.5		4.73	1.15	2.534	49.3	100	94
12	Diltiazem	415		7.7	3.65	2.06	2.210	59.1	90	91
13	Famotidine	338		7	0.26	−0.44	0.559	173.3	40	38
14	Furosemide	331	3.9		1.90	−0.69	1.807	122.6	65 (62–67)	78
15	Ganciclovir[i]	255	9.4	2.2	−2.54		−1.560	139.3	5	11
16	Hydrochlorothiazide	298	7, 9.2		−0.36	−0.12	0.584	118.4	70	54
17	Imipramine	280		9.5	5.04	2.40	2.600	6.5	100	96
18	Indomethacin	358	4.5		4.18	1.00	3.019	68.5	100	95
19	Ketorolac	255	3.5		1.62	−0.27	1.477	59.3	95	84
20	Lisinopril	406	1.7, 3.3, 11.1	7	−1.69		−0.959	133	25	19
21	Metolazone[j]	366	9.7		2.02	1.84	1.684	94.6	64	81
22	Naproxen	230	4.2		2.82	0.23	2.131	46.5	100	92
23	Norfloxacin	319	6.3	8.8	−0.97	−0.81	0.978	74.6	65	73
24	Propranolol	259		9.5	2.75	1.26	2.119	41.5	95	92
25	Ranitidine	314		2.3, 8.2	0.63	−0.29	0.613	108.3	50	57
26	Terbutaline	225	10.1, 11.2	8.8	0.48	−1.35	0.332	72.7	53 (25–80)	61
27	Timolol	316		8.8	1.58	0.03	0.796	79.7	90	69
28	Verapamil	455		8.9	4.47	2.66	3.004	64.0	90	95
Test set										
29	Atenolol[k]	266		9.6			−0.292	84.6	44	43
30	Benzyl penicillin[j]	334	2.8				−0.600	88.9	30	35
31	Cefuroximeaxetil[j]	510	2.45				1.210	188.8	44	49
32	Cimetidine	252		6.8			0.231	88.9	64	54
33	Enalapril[j,k]	376	3	5.5			−0.337	97.8	60	39
34	Furosemide[k]	331	3.9				1.599	122.6	61	74
35	Hydrochlorothiazide[k]	298	7, 9.2				0.754	118.4	70	58
36	Ketoprofen	254	4.8				1.797	54.4	100	88
37	Labetalol[k]	328	8.7	7.4			1.900	95.6	95	84
38	Metoprolol	267		9.7			0.873	50.7	95	77
39	Nifidipine	346					1.740	132.5	90	75
40	Predinisolone[k]	360					1.648	94.8	99	81
41	Propranolol[k]	259		9.5			1.452	41.5	95	86
42	Sulindac[j,k]	356		4.5			1.438	57.7	90	84

[a] Molecular weight (MW) obtained from Veber et al. (2002).
[b] pK_a values were obtained from Sugano et al. (2002) and Williams and Lemke (2002).
[c] Calculated log P values were obtained from program Clog P v4.0.
[d] Experimental partition coefficient values at pH 7.4 obtained from Kansy et al. (2001) and Zhu et al. (2002).
[e] Highest value of log k'_{IAM} among pH 4.5–7.4 determined by HPLC (n = 3, S.D. < 0.03).
[f] Polar surface area (PSA) obtained from Veber et al. (2002).
[g] %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).
[h] %HOA calculated using coefficients of model 5 in Table 3.
[i] pK_a values were obtained from http://www.boomer.org/pkin/PK03/PK2003343.html.
[j] MW and PSA values were obtained from program Cerius^2 ADME module v4.10.
[k] log k'_{IAM} values were obtained from previously studied values (Caldwell et al., 1998; Chan et al., 2005; Yen et al., 2005).

their retention time (>30 min). IAM capacity factor of these compounds were determined by extrapolating to zero acetonitrile percentage using linear regression. Excellent linearity ($r > 0.99$) was found over the whole eluent composition range when $\log k'_{IAM}$ plotted against %v/v acetonitrile in mobile phase.

3.2. Relationship between IAM capacity factor and partitioning in n-octanol/water system

The comparison between $\log k'_{IAM}{}^{7.4}$ and lipophilicity parameter $\log P$ was made for the nine acidic compounds (ACs) (compound nos. 4, 16, 17, 20, 22–24, 27, 34 from Table 2). At a pH value more than one unit away from pK_a ($pK_a + 1$ for acids), an ionizable compound can be assumed to exist in its total ionized form. The pK_a values of the ACs considered span the range of 3–5.2, so the degree of ionization of all the compounds can be assumed to be complete at pH 7.4. The $\log k'_{IAM}{}^{7.4}$ values for the above compounds are well predicted from the respective $\log P$ values using Eq. (1). Numbers in parentheses account for the standard error of the regression coefficients.

$$\log k'_{IAM}{}^{7.4} = 0.50(\pm 0.09)\ \log P - 0.48(\pm 0.26),$$
$$n = 9; \quad R^2 = 0.8207; \quad s = 0.31 \qquad (1)$$

Eq. (1) indicates that the ranking order on the IAM for ACs is governed by their intrinsic lipophilicity and is not affected by the presence of an electric charge on the molecule. Therefore it could be very useful to compare the $\log k'_{IAM}$ values of AC with the $\log k'_{IAM}$ values of non ionizable neutral compounds (NCs) (compound nos. 1–3, 6, 8–10, 15, 19, 28–30 from Table 2). The $\log k'_{IAM}{}^{7.4}$ values of NCs used to derive this relationship show good relationship with their $\log P$ values (Eq. (2)). A poor relationship was found between $\log k'_{IAM}{}^{7.4}$ and $\log P$ values after considering NCs and ACs together as shown in Eq. (3). This relationship does not allow inferring that the acidic compounds/phospholipids interactions are uniquely lipophilicity-based. Good correlation in Eq. (1) could be because of (a) either attractive or repulsive additional forces occurring in constant extent for all compounds and/or (b) decrease in mobile phase strength at high pH (7.4) and therefore decrease in $\log k'_{IAM}$ in constant manner.

$$\log k'_{IAM}{}^{7.4} = 0.58(\pm 0.06)\ \log P + 0.05(\pm 0.14),$$
$$n = 12; \quad R^2 = 0.8975; \quad s = 0.26 \qquad (2)$$

$$\log k'_{IAM}{}^{7.4} = 0.46(\pm 0.08)\ \log P - 0.01(\pm 0.20),$$
$$n = 21; \quad R^2 = 0.6483; \quad s = 0.44 \qquad (3)$$

Table 2
Physicochemical properties of compounds to study molecular factors influencing retention in IAM

No.	Compound	pK_a[a]		$\log P$[b]	$\log k'_{IAM}$ (pH 7.4)[c]	$\log k'_{IAM}$ (pH 4.5)[c]
		HA	HB+			
1	Acetanilide		0.5	1.16	0.480	
2	Acetoaminophen	9.7		0.49	0.346	
3	Acetophenone			1.58	0.760	
4	Acetyl salycylic acid	3.5		1.02	−0.042	0.400
5	Amiloride		8.7	−0.55	0.862	
6	Antipyrine		1.5	0.20	0.222	
7	Atenolol		9.6	−0.11	0.039	0.039
8	Benzamide			0.64	0.121	
9	Carbamazepine			1.98	1.392	
10	Chlorobenzene			2.89	1.584	
11	Chlorothiazide	6.8, 9.5		−0.24	−0.021	
12	Chlorpromazine		9.3	5.80	3.218	
13	Chlorthalidone	9.4		0.45	1.023	1.161
14	Clonidine		8.3	1.73	1.181	
15	Corticosterone			2.10	1.694	
16	Diclofenac	4.5		4.73	1.708	2.534
17	Furosemide	3.9		1.90	0.915	1.807
18	Hydrochlorothiazide	7, 9.2		−0.36	0.525	0.584
19	Hydrocortisone			1.61	1.303	
20	Ibuprofen	5.2		3.68	1.329	2.033
21	Imipramine		9.5	5.04	2.600	
22	Indomethacin	4.5		4.18	1.928	3.019
23	Ketoprofen	4.8		2.76	0.997	1.797
24	Ketorolac	3.5		1.62	0.430	1.477
25	Metolazone	9.7		2.02	1.522	1.684
26	Metoprolol		9.7	1.35	0.873	
27	Naproxen	4.2		2.82	0.787	2.131
28	Napthalene			3.30	2.149	
29	Nifedepine			3.41	1.740	
30	Nimodepine			4.18	2.352	
31	Procaine		8.8	2.30	0.886	
32	Propranolol		9.5	2.75	2.119	
33	Ranitidine		2.3, 8.2	0.63	0.613	
34	Salycylic acid	3.0, 13.4		2.19	0.080	0.319
35	Terbutaline	10.1, 11.2	8.8	0.48	0.332	
36	Timolol		8.8	1.58	0.796	
37	Verapamil		8.9	4.47	3.004	

[a] pK_a values were obtained from Sugano et al. (2002) and Williams and Lemke (2002).
[b] Calculated $\log P$ values were obtained from program Clog P v4.0.
[c] Capacity factors determined at pH 4.5 and/or 7.4 ($n = 3$, S.D. < 0.03).

Similarly comparison between $\log k'_{IAM}{}^{7.4}$ and lipophilicity parameter $\log P$ was made for the 12 basic compounds (BCs) (compound nos. 5, 7, 12, 14, 21, 26, 31–33, 35–37 from Table 2). At a pH value less than one unit away from pK_a ($pK_a - 1$ for base), an ionizable compound can be assumed to exist in its total ionized form. The pK_a values of the BCs considered span the range of 8.2–9.6, so the degree of ionization of all the compounds can be assumed to be complete at pH 7.4. The $\log k'_{IAM}{}^{7.4}$ values for the basic compounds are well predicted from the respective $\log P$ values using the following equation:

$$\log k'_{IAM}{}^{7.4} = 0.49(\pm 0.06)\ \log P + 0.34(\pm 0.16),$$
$$n = 12; \quad R^2 = 0.8800; \quad s = 0.39 \tag{4}$$

Eq. (4) further indicates that the ranking order on the IAM for basic compounds is governed by their intrinsic lipophilicity and is not affected by the presence of an electric charge on the molecule. The inclusion of NCs in Eq. (4) generates a new Eq. (5), which is statistically similar to Eq. (4). This relationship indicates that the interaction between basic compounds/phospholipids is uniquely lipophilicity-based. It also confirms that phospholipids can counteract the positive charges on the solutes.

$$\log k'_{IAM}{}^{7.4} = 0.51(\pm 0.04)\ \log P + 0.23(\pm 0.11),$$
$$n = 24; \quad R^2 = 0.8778; \quad s = 0.33 \tag{5}$$

$$\log k'_{IAM}{}^{4.5} = 0.59(\pm 0.07)\ \log P + 0.06(\pm 0.18),$$
$$n = 21; \quad R^2 = 0.7930; \quad s = 0.4 \tag{6}$$

From the results obtained so far, it appears that IAM affinity scale is distinctive from $\log P$ scale when considering diverse compounds. Referring to our previous work (Kotecha et al., 2007), compounds with negative charge at physiological pH 7.4, showed higher retention at acidic pH (e.g. 4.5) compare to more traditional pH 7.4. By replacing $\log k'_{IAM}{}^{7.4}$ values with $\log k'_{IAM}{}^{4.5}$ values for ACs in Eq. (3), generates new relationship Eq. (6) with better correlation. This indicates that IAM phase partially counteracts the negative influence of the electric charges on the solutes when chromatography is operated at suitable pH.

3.3. Non-linear regression analysis between human oral absorption and physicochemical descriptors

The $\log k'_{IAM}$ (at pH 7.0 or 7.4) is often considered as effective molecular descriptor, indicative of the potential absorption properties of drugs (Stewart et al., 1998; Genty et al., 2001; Osterberg et al., 2001; Chan et al., 2005). The possible correlation between $\log k'_{IAM}{}^{7.4}$ of the training set compounds from Table 1 vs. the corresponding %HOA was checked using empirical sigmoid function Eq. (7). Sigmoidal relationships between HOA and $\log k'_{IAM}$ were observed in present study, which is in agreement with previous observations (Raevsky et al., 2000; Stenberg et al., 2001; Sugano et al., 2002; Matsson et al., 2005).

$$Y = \frac{100}{1 + 10^{-(A1+(A2\times X))}} \tag{7}$$

Here, Y is the %HOA of a series of compounds; X is the independent variable (e.g. $\log k'_{IAM}$). A1 and A2 are regression coefficients obtained by non-linear regression. As expected most lipophilic drugs showed higher %HOA, however various outliers were observed and no significant correlation was found between two series of values, model 1 in Table 3. For number of drugs mainly

Table 3
Regression coefficients and statistical criteria for relationship $\%HOA = 100/(1 + 10^{-(A1+(A2\ \log k'_{IAM})+(A3|pK_a - pH|)+(A4\times MW)+(A5\times PSA)+(A6\ \log D^{7.4}_{oct})+(A7\ \log P)})$

Model	Regression coefficients							Statistical criteria		
	A1[a]	A2	A3	A4	A5	A6	A7	n	R^2	s
1	−0.046 ± 0.085 (0.591)	0.566 ± 0.113 (≤0.001)	–	–	–	–	–	28	0.7403	15
2	−0.140 ± 0.111 (0.221)	0.551 ± 0.109 (≤0.001)	0.069 ± 0.057 (0.233)	–	–	–	–	28	0.7574	14
3	−0.181 ± 0.071 (0.017)	0.526 ± 0.720 (≤0.001)	–	–	–	–	–	28	0.8566	11
4	0.014 ± 0.313 (0.964)	0.506 ± 0.077 (≤0.001)	–	0.014 ± 0.113 (0.905)	−0.239 ± 0.129 (0.077)	–	–	28	0.8760	11
5	0.412 ± 0.171 (0.024)	0.407 ± 0.063 (≤0.001)	–	–	−0.491 ± 0.139 (0.002)	–	–	27	0.9001	9
6	−0.158 ± 0.076 (0.048)	0.507 ± 0.074 (≤0.001)	–	–	–	–	–	27	0.8454	11
7	0.416 ± 0.092 (≤0.001)	–	–	–	–	0.398 ± 0.099 (≤0.001)		24	0.6008	15
8	0.110 ± 0.060 (0.796)	–	–	–	–	–	0.280 ± 0.046 (≤0.001)	28	0.7887	13

[a] Regression coefficient ± S.E. (p-value of coefficient).

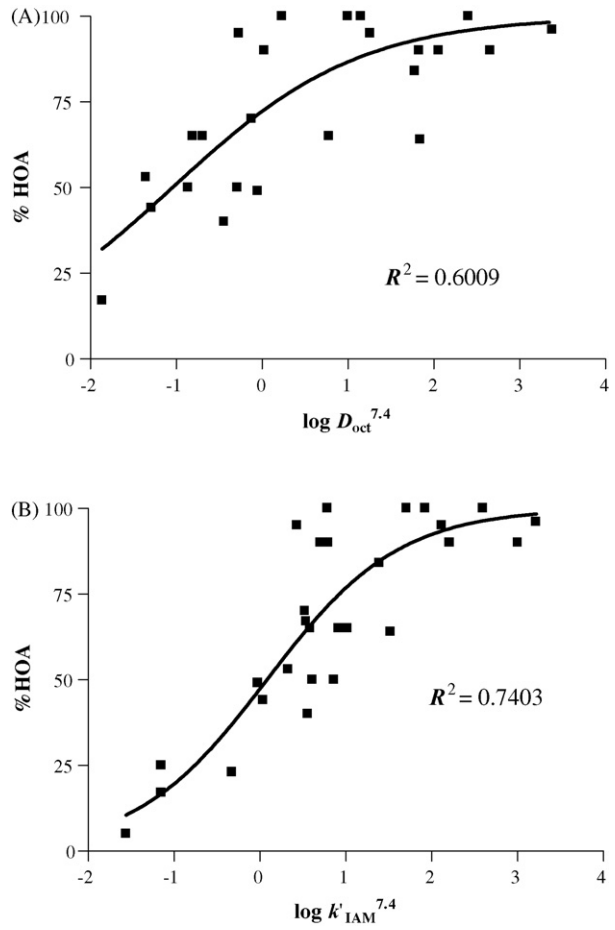

Fig. 1. (A) Plot of %HOA and experimental octanol/water distribution coefficient at pH 7.4 and (B) similar to (A) with $\log k'_{IAM}{}^{7.4}$ for training set.

acidic in nature (e.g. furosemide, ketorolac), the $\log k'_{IAM}$ values measured at pH 7.4 leads to an underestimation of the fraction absorbed. For comparison, the predictive value of octanol/water distribution coefficients at pH 7.4 was significantly lower than $\log k'_{IAM}{}^{7.4}$ (Fig. 1). According to pH partition theory, permeability of weak electrolytes is affected by the pH conditions following the change in compound dissociation (Hogben et al., 1959). Therefore we quantitatively analyzed HOA, using the absolute value of the difference between the p*K*a of the drug and experimental pH (7.4), $|pK_a - pH|$. This indirectly helps for correcting fraction ionized at experimental pH. The regression coefficients and statistical results for this equation, model 2 in Table 3 indicated no significant ($p > 0.05$) improvement in relationship between HOA and $\log k'_{IAM}{}^{7.4}$ after considering $|pK_a - pH|$ values.

To mimic an environment, which more closely resembles the conditions encountered as the substance moves through the gastrointestinal (GI) tract and knowing the importance of pH conditions to counteract the negative influence of phospholipids on retention of acidic compounds, capacity factor measurements were made at varying pH values, from pH 4.5–7.4. The highest $\log k'_{IAM}{}^{4.5-7.4}$ value was taken into account for each drug to determine correlation. A reasonably good relationship (model 3 in Table 3) was obtained between the two sets of data. There was substantial decrease in residual values for acidic drugs.

The transport of drug molecules across the biomembranes is a complex process and influenced by many factors. With an aim to further improve the correlation between $\log k'_{IAM}{}^{4.5-7.4}$ and HOA, HOA was quantitatively analyzed using the physicochemical parameters (descriptors) of these drugs; MW and PSA in conjunction with $\log k'_{IAM}{}^{4.5-7.4}$. The physicochemical descriptor values for these 28 drugs are presented in Table 1. The inter-correlation of these descriptors was checked and found cross-correlation in R^2 as shown in Table 4. None of the pair of descriptors showed good correlation (>0.8). The fourth regression model in Table 3 was built using $\log k'_{IAM}{}^{4.5-7.4}$, MW and PSA. For practical reason MW and PSA values were converted in to fraction before applying non-linear regression analysis. But the results showed that no other variable except $\log k'_{IAM}{}^{4.5-7.4}$ met the 0.05 significance level for entry into the model.

Table 4
Correlation matrix of descriptor values

	$\log k'_{IAM}{}^{4.5-7.4}$	MW	PSA
$\log k'_{IAM}{}^{4.5-7.4}$	1		
MW	0.34	1	
PSA	−0.56	0.11	1

Further, to confirm the non significant role of MW and PSA in explaining factors affecting oral absorption, a set of bi-plots were plotted for drugs under investigation by plotting (a) MW vs. HOA and (b) PSA vs. HOA (Fig. 2). MW did not show any trend to explain its effect on HOA. But increase in PSA results into negative contribution to HOA. Closer inspection shows that HOA trends upward with decreasing PSA and moderate to high absorption (>50%) observed at PSA value <100 Å^2. The %HOA value of bretylium (compound no. 4, Table 1) with PSA 13 Å^2 was found to lie below the trend line. Bretylium does not follow the PSA criterion for good oral absorption, i.e. completely absorbed molecules (%FA > 90%) exhibited PSA values of ≤60 Å^2 (Palm et al., 1997). After deleting bretylium from the training set, non-linear regression of HOA against $\log k'_{IAM}{}^{4.5-7.4}$

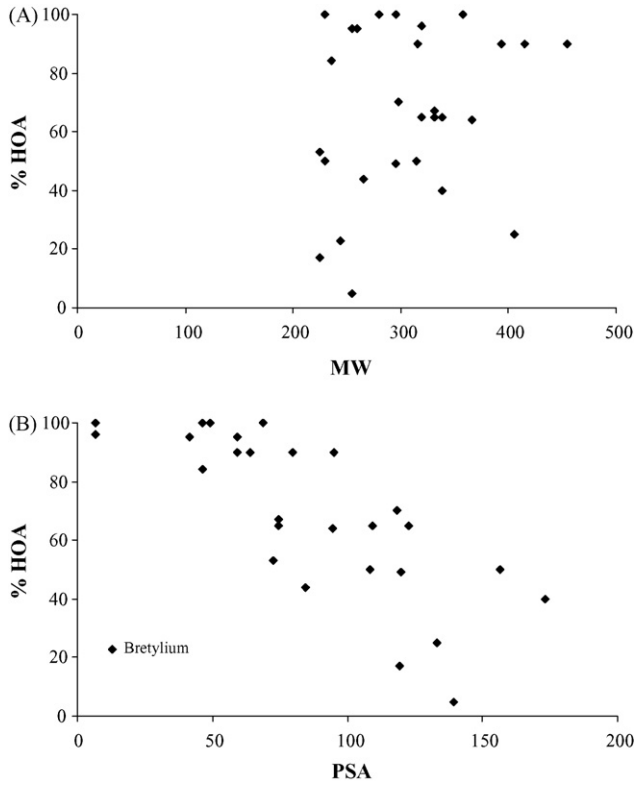

Fig. 2. The bi-plots (A) MW vs. %HOA and (B) PSA vs. %HOA demonstrating the relevance of each descriptor to explain oral absorption for the compounds under investigation (training set).

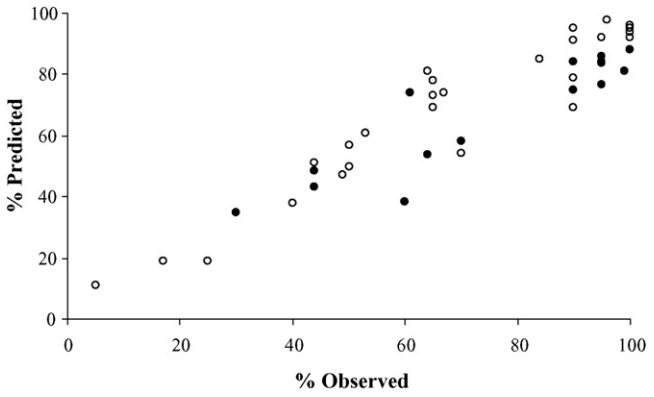

Fig. 3. Plot of %observed vs. %predicted HOA by model 5. (○) drugs used in regression analysis (training set); (●) drugs used in test set.

and PSA showed that both the descriptors were statistically significant to explain factors affecting HOA (model 5 in Table 3). The $\log k'_{IAM}{}^{4.5-7.4}$ along with H-bonding parameter (PSA) gave an enhanced correlation as compared to $\log k'_{IAM}{}^{4.5-7.4}$ alone (model 6 in Table 3). A graphical comparison of the calculated HOA values based on model 5 and observed HOA is given in Fig. 3.

4. Discussion

4.1. Relationship between IAM capacity factor and partitioning in n-octanol/water system

Ionizable drugs in the given data set are partly or fully in ionized form under experimental conditions. The retention behavior of the neutral and ionized forms was quite different on the IAM column because an electrostatic force is involved in the interactions between the ionized form and the phospholipids membrane, but it was not the case with neutral compounds.

A correlation between $\log k'_{IAM}{}^{7.4}$ and $\log P$ was established by considering the NCs and ACs. It was obvious that there were two subsets in the ACs group, with one subset containing a carboxylic group (–COOH) and the other without such group. The regression line of ACs with –COOH was almost parallel to, but did not overlap, that of NCs and ACs without –COOH (Fig. 4). This indicates that the carboxylic group is an important factor affecting the retention of ACs, and it interferes with the retention markedly. It was suggested that the structural feature of –COOH function directly linked to an aromatic ring, caused a disturbance in the lipophilic interaction with phospholipids (Barbato et al., 1997). This is little different from our observation as few compounds in our data set consist –COOH group in side chain. Our result indicates that the position of the –COOH group does not seem to be important as far as occurrence of disturbance is concerned. This could be because of the different stationary phase used in their experiments. Lipophilic interaction of naproxen, ketorolac and furosemide with phospholipids was sensitive to the presence of the ionized group; the $\log k'_{IAM}$ values at pH 4.5 were practically higher to those at pH 7.4. This result confirms that for these compounds, higher the ionization of the molecule, higher the disturbance of the electric charge on the lipophilic interaction. On the other hand weak acids chlorothiazide, chlorthalidone, hydrochlorothiazide and metolazone in our data set all without carboxylic group in their structures had comparable retention with NCs. A good relationship was achieved between $\log k'_{IAM}$ and $\log P$ for ACs (with –COOH) and NCs together after determining $\log k'_{IAM}$ of ACs at pH other than 7.4 (e.g. 4.5). Acidic drugs clearly form a subgroup that behaves differently in IAM chromatography at acidic pH with improvement in overall relationship. These observations reveal 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. 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 unionized form is supposed to be able to partition significantly into the lipid phase (Hansch et al., 1987).

The good relationship between 12 basic compounds and 12 neutral compounds evidenced that there was an attractive extra interaction for BCs (Fig. 5). This phenomenon was already observed for basic molecules (Austin et al., 1995; Barbato et al., 1996) which also showed attractive extra-interactions with the charged moiety of phosphatidylcholine. Five secondary amines (atenolol, metoprolol, propranolol, terbutaline and timolol) and three tertiary amines (chlorpromazine, imipramine and verapamil) lay close to the NCs regression line, which indicates that the phospholipids membrane is able to mask the effect of the electronic charge of the amino group on their retention. Phospholipids interact with their protonated amines to the same extent as neutral isolipophilic compounds. It appears that a secondary and tertiary amino group is important for stronger interactions with the phospholipids membrane. This relationship suggests that the behavior of ionizable drugs in the body is controlled by the interaction of both neutral and ionized form with the biological membranes. The "pH piston hypothesis" describes how the partition of ionizable compounds in biomembranes is strongly affected by electrostatic and/or hydrogen bond interactions with phospholipids (Avdeef et al., 1998). The GI-tract

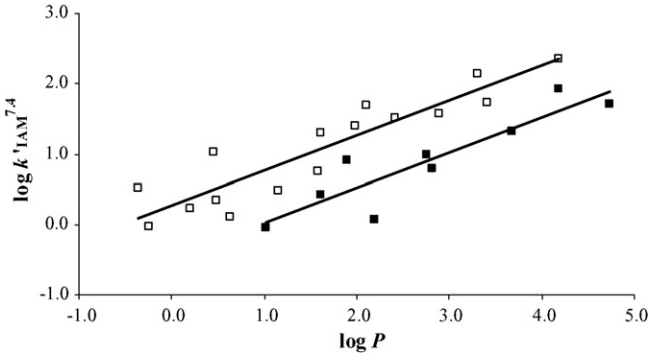

Fig. 4. Relationship between IAM chromatographic parameter ($\log k'_{IAM}{}^{7.4}$) and $\log P$. (□) NCs and ACs where ACs without –COOH group in their structures; (■) ACs with –COOH group in their structures.

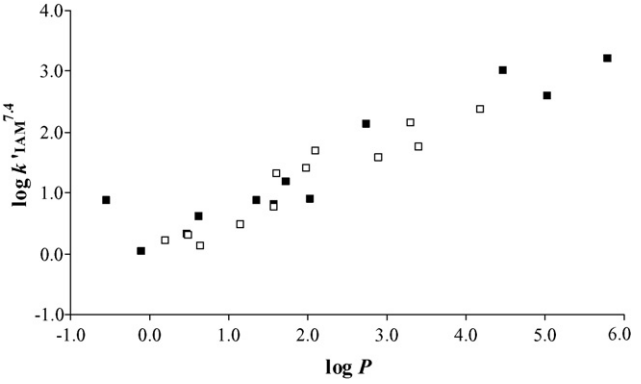

Fig. 5. Relationship between IAM chromatographic parameter ($\log k'_{IAM}{}^{7.4}$) and $\log P$ of NCs (□) and BCs (■).

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.

4.2. Non-linear regression analysis between human oral absorption and physicochemical descriptors

Upon the basis of our examination of the relevant literature, the most appropriate factors to be considered in a passive absorption model are lipophilicity, hydrophilicity, size and degree of ionization. To assess the value of our approach we compared our data with experimental and theoretical approaches which have been proposed to predict drug absorption. The octanol/water distribution coefficient ($\log D_{oct}^{7.4}$) is widely used for prediction of GI-tract absorption of ionizable substances. Comparison of models indicate that $\log k'_{IAM}{}^{7.4}$ is performing better than $\log D_{oct}^{7.4}$ to predict HOA (model 7 in Table 3). In particular, chlorothiazide, chlorthalidone and metolazone are largely overestimated in octanol/water system. This is more likely due to the inadequate hydrogen bond acidity component of octanol. Indeed, these three substances have a large number of strong hydrogen bond acceptor groups. In addition, experimental determination of $\log D$ vs. pH is rather labor-intensive and therefore not suitable for high-throughput. Good correlation between $\log P$ and $\log k'_{IAM}{}^{4.5/7.4}$ for acidic and basic compounds, respectively leads to correlate $\log P$ and %HOA for training compound set (model 8 in Table 3). However, ciprofloxacin and norfloxacin are largely underestimated because $\log P$ does not account for modification in hydrophobicity of ionizable compounds at varying pH and ionic interaction with biological membrane.

$\log k'_{IAM}{}^{4.5-7.4}$ parameter is assumed to quantify hydrophobic interactions and ionic interactions with phospholipids. We selected PSA and MW as descriptors in addition to $\log k'_{IAM}{}^{4.5-7.4}$ for model development. The correlation of the chosen variables with each other must be taken into account when building a model. Fig. 6 is a set of 2D plots of the given dataset, plotting (a) MW vs. $\log k'_{IAM}{}^{4.5-7.4}$, (b) MW vs. PSA and (c) $\log k'_{IAM}{}^{4.5-7.4}$ vs. PSA. Generally MW is shown to have a hyperbolic bounded relationship with $\log P$ (Egan et al., 2000). Closer inspection shows that for $\log k'_{IAM}{}^{4.5-7.4} > 0$, MW trends upward with increasing $\log k'_{IAM}{}^{4.5-7.4}$ but we do not have enough data point to show hyperbolic relationship, i.e. MW begins to trend upward with decreasing $\log k'_{IAM}{}^{4.5-7.4}$. MW is generally shown to increase as PSA increases (increase in number of nitrogen and oxygen atoms in molecules and thereby increase in attached hydrogen atoms) (Egan et al., 2000), but this is not observed in present data set. Decrease in $\log k'_{IAM}{}^{4.5-7.4}$ with increase in PSA is indicative of compounds' H-bonding ability and preference for aqueous mobile phase. This is the only relationship that gives better correlation (Table 4).

Non-linear regression between all the descriptors ($\log k'_{IAM}{}^{4.5-7.4}$, PSA and MW) and HOA showed that MW and PSA were not statistically significant to explain HOA ($p > 0.05$). The conclusion about MW contradicts the results published; they observed MW dependence of the sigmoidal lipophilicity–permeability relationship in their data (Camenisch et al., 1998). However, the demonstrated relationships of MW to both PSA and $\log k'_{IAM}{}^{4.5-7.4}$ (Fig. 6A and B), the sigmoidal relationship between PSA and HOA (Fig. 2A), and the known physical importance of H-bonding and lipophilicity to membrane permeation, all support the conclusion that $\log k'_{IAM}{}^{4.5-7.4}$ and PSA are the most relevant descriptors. Fig. 7 is a 3D plot of MW on the PSA–$\log k'_{IAM}{}^{4.5-7.4}$ axes for the given

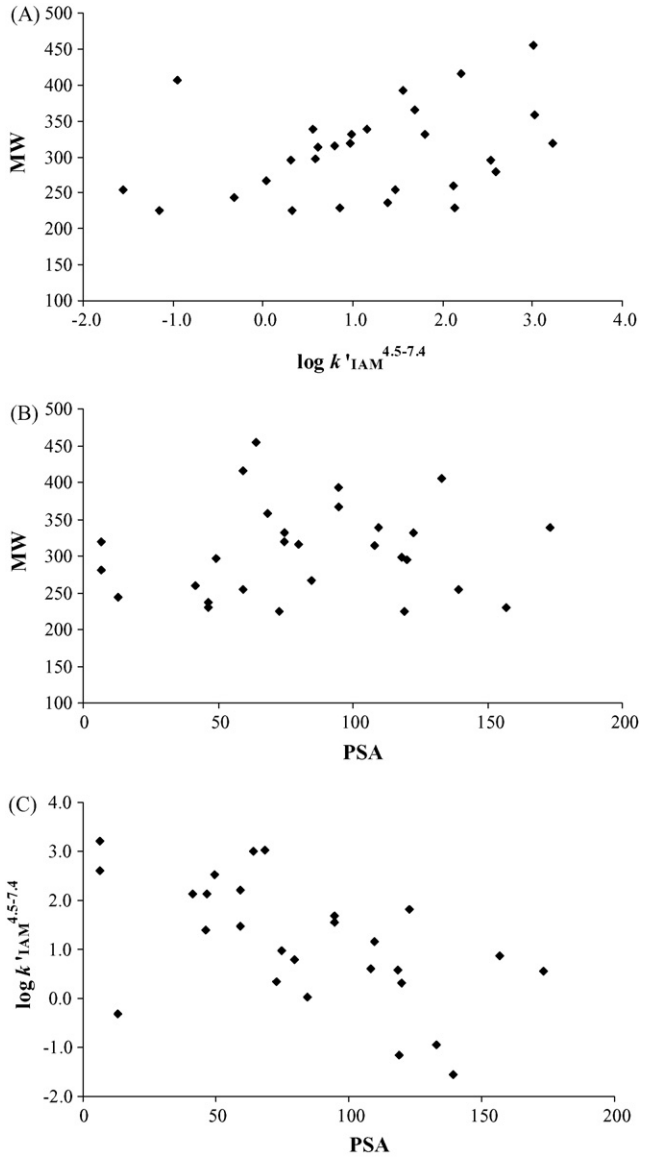

Fig. 6. Three bi-plots (A) $\log k'_{IAM}{}^{4.5-7.4}$ vs. MW, (B) PSA vs. MW and (C) PSA vs. $\log k'_{IAM}{}^{4.5-7.4}$ demonstrating the interrelationships between MW and the other two descriptors for the compounds under investigation (training set).

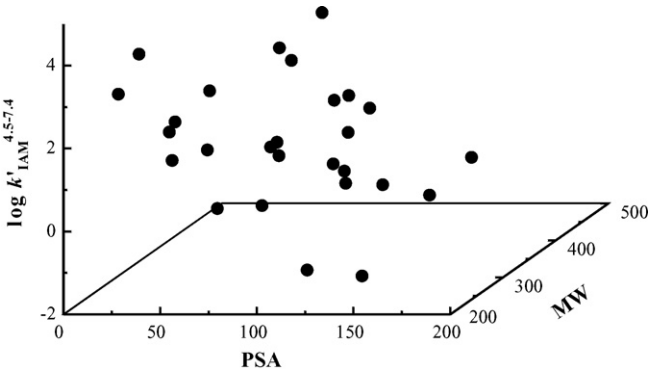

Fig. 7. 3D plot of MW on PSA and $\log k'_{IAM}{}^{4.5-7.4}$ for training set.

dataset. At low PSA and high $\log k'_{IAM}{}^{4.5-7.4}$, MW is in the range 230–455 and at high PSA and low $\log k'_{IAM}{}^{4.5-7.4}$, MW is in the range 225–406; both MW ranges are almost similar and considered acceptable for small-molecule drug design. This suggests that the information relevant to drug absorption is sufficiently end coded in lipophilicity plus PSA, without explicit reference to MW.

The $\log k'_{IAM}{}^{4.5-7.4}$ together with the PSA gave an enhanced correlation (model 5 in Table 3) as compared to using $\log k'_{IAM}{}^{4.5-7.4}$ alone (model 6 in Table 3) after removal of bretylium from the dataset for known reason. This is in agreement with previous works that suggests hydrogen bond donors and hydrogen bond acceptors or polar molecular surface are good descriptors with which to model human intestinal absorption (Palm et al., 1997; Clark, 1999; Raevsky et al., 2000; Stenberg et al., 2001). Different training sets by Abraham descriptors show that increasing the volume (hydrophobic part) and decreasing the polarity (PSA) of a compound can increase human intestinal absorption. Introducing solubility, octanol/water partition coefficient, MW and pK_a terms did not improve the regression results (Zhao et al., 2001). The negative dependence of %HOA on the PSA can be rationalized based on the inverse relation that exists between the diffusion coefficients of a solute and its PSA. Because a cell membrane is comprised of hydrophilic and lipophilic regions, a molecule that passes through a cell membrane through the transcellular pathway needs to penetrate both hydrophilic and hydrophobic environments. As a result both hydrophilic and lipophilic properties of a drug should be taken into account when predicting drug absorption. Thus the PSA value was found to be second important descriptor for predicting drug absorption as negatively correlated to intestinal absorption. PSA is a measure of the proportion of molecule's mass that is hydrophilic. As the PSA value increases, the hydrophilic character of a drug molecule increases. It is difficult for a drug molecule with mainly hydrophilic structure to penetrate the outer layer (phospholipids layer) of the cell membrane by transcellular diffusion. Thus, the intestinal absorption decreases as the PSA value increase.

The PSA criterion for poor absorption, i.e. compound with PSA $\geq$ 140 Å^2 should exhibit fraction absorbed <10% (Palm et al., 1997), seems robust but it is not immune from making false positive assessments. A good example of this is bretylium, which has a very low PSA value but it is poorly absorbed. This indicates that a PSA of <140 Å^2 is a necessary, but not sufficient criterion for oral absorption. The cause of the poor absorption in case of bretylium, is the presence of the positively charged quaternary nitrogen which inhibits partitioning into the intestinal membranes. But, in case of amiloride, famotidine and furosemide PSA helped to avoid over prediction. These drugs have relatively high PSA and moderate oral absorption. But simple model ($\log k'_{IAM}{}^{4.5-7.4}$ vs. %HOA) predicts oral absorption on higher side. Multiple regression of $\log k'_{IAM}{}^{4.5-7.4}$ with PSA results into accurate prediction of their oral absorption (Table 1). Final model (model 5) exists with no outlier (data not shown) but still one drug timolol shows high residual values (>20%). It is perhaps related to the diversity in intestinal absorption data in the literature (Dollery, 1999; Sugano et al., 2001; Wohnsland and Faller, 2001; Zhu et al., 2002).

The model 5 (Table 3) was tested for its accuracy in predicting HOA using structurally diverse drugs with varying physicochemical properties (test set, Table 1). There observed oral absorption was in the range of 30–100% which is generally acceptable range for drug development program. The log k'_{IAM} values of some of the drugs were taken from the literature considering their experimental conditions for determination of IAM capacity factors (compound nos. 29, 33–35, 37 and 40–42, Table 1). Out of these, four drugs (compound nos. 29, 34, 35 and 41) were already considered in training set. The graphical result of their oral absorption prediction is shown in Fig. 3. The predicted absorption values are in agreement with the corresponding observed absorption values for most of the test drugs (Table 1). These results indicate that it is possible to predict the intestinal absorption of drugs of all sorts by the use of regression analysis including the parameters which indicate the ionic interaction between the drug and the biomembranes in addition to lipophilicity and hydrogen bonding capacity.

4.3. Published relations of IAM capacity factors with human oral absorption

Number of approaches was utilized to improve correlation between IAM capacity factor and fraction absorbed in humans particularly using molecular descriptors. The chromatographic capacity factors (k'_{IAM}) were determined as a function the pH and composition of mobile phase, and were corrected for the molar volume of the solute (k'_{IAM}/MW^n). The correlation between k'_{IAM}/MW^n and human fraction of intestinal absorption (Fa) was highest ($R = 0.925$) when measured at 20% acetonitrile (pH 5.5) with the power function $n = 2.5$ using sigmoid function (Yoon et al., 2004). But the direct correlation between k'_{IAM} and Fa was not plotted using sigmoid function; it was plotted on log–log scale with linear regression. 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 using linear regression (Yen et al., 2005). In their work, there was no explanation about selection of pH 5.4 for determination of IAM capacity factor. And also inter-correlation was not checked for descriptors used in their analysis.

In this context previously we quantitatively analyzed $\log k'_{IAM}$ of structurally diverse drugs using pH partition theory and presented good correlation ($R^2 = 0.8566$) with HOA (Kotecha et al., 2007), which is better than the published relationship. Results of present study indicate that $\log P/D$ is no more good lipophilicity marker compare to $\log k'_{IAM}{}^{4.5-7.4}$, because they do not support ionic interaction of drugs with phospholipids membrane which actually takes place in oral absorption. Further, to still improve the correlation, we also studied $\log k'_{IAM}{}^{4.5-7.4}$ in conjunction with other fundamental variables of membrane permeability (e.g. MW, PSA) and found PSA as a second important descriptor for prediction of HOA. The present study will be helpful in drug development by its contribution to more reliable prediction of HOA particularly for drugs absorb via passive transcellular path way. Future studies should cover high MW (MW > 600) range in order to understand the contribution of size in passive transcellular diffusion.

5. Conclusion

The relationship between $\log k'_{IAM}{}^{7.4}$ and $\log P$ suggests that retention of ACs containing a carboxylic acid group is strongly influenced by dissociation of their carboxylic group. There is a repulsive interaction between the ionized forms of carboxylic compounds and the polar head of phospholipids so that the retention of the former was impaired. Nevertheless, the retention of BCs does not seem to be significantly affected by ionization, attractive interactions play an important role in the retention. So, when ionized compounds were included in the compounds set, carboxylic acids and basic compounds have to be treated separately. We also described the development of a general experimental and computation model for human passive oral absorption. The descriptors found suitable to successfully explain oral absorption were $\log k'_{IAM}{}^{4.5-7.4}$ and PSA. The success was based on consideration of the physical processes

involved in membrane permeability and fact that PSA provides a reference point for $\log k'_{IAM}$. The resultant model has several advantages. The descriptors PSA and $\log k'_{IAM}$ are physically meaningful and easily related to structure, making them relatively straightforward for a medicinal chemist to interpret. Implementation of method enables rapid screening of membrane penetration properties of drug with respect to its lipophilicity, charge state and polarity. The results of this study show that the $\log k'_{IAM}{}^{4.5-7.4}$ value obtained with the developed IAM-HPLC in conjunction with PSA are good predictors of the oral absorption of passive transcellularly absorbed drugs in humans. Moreover, the proposed method allowed obtainment of good results not only for highly absorbed hydrophobic compounds, but also for poorly absorbed compounds. The only exception was bretylium, whose poor in vitro–in vivo correlation was explained by its chemical nature.

Acknowledgements

The authors are thankful to Dr. M.C. Gohel (Principal and Head, Department of Pharmaceutics and Pharmaceutical Technology, L.M. College of Pharmacy) and Dr. B.N. Suhagia (Professor, Department of Pharmaceutical Chemistry, L.M. College of Pharmacy) for their assistance and fruitful discussions during the conduct of this study.

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