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IMMOBILIZED ARTIFICIAL MEMBRANE CHROMATOGRAPHY: A USEFUL TOOL FOR PREDICTING MEMBRANE PERMEABILITY

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ABSTRACT

The potential of immobilized artificial membrane chromatography (IAM) chromatography to predict intestinal and blood brain barrier (BBB) permeability was investigated utilizing retention indices and other permeability factors compiled from literatures such as the lipophilicity factor, logarithms of octanol/water partition coefficient (log P) and blood/brain partition coefficient (log BB). For this purpose, the separation of a diverse structures and functions set of 21 compounds was studied on stationary immobilized artificial membrane chromatography and their chromatographic retention factors; namely aqueous capacity factor (log K_{IAMW}) were determined. The aqueous capacity factors of the compounds were correlated with their log P and log BB. Linear correlation was obtained between log K_{IAMW} and log P of the compounds ($r > 0.95$). Log K_{IAMW} values of the compounds also showed an acceptable correlation with log BB ($r > 0.76$). Inclusion of the molecular size of the compounds with log K_{IAMW} data had negative effect on correlations when the data plotted vs. log BB values. For a subset of 8 compounds, log K_{IAMW} showed better correlation with log % of human absorption than did log P. These results show that the aqueous capacity factor, log K_{IAMW} seems to provide a valuable assessment of drug permeability through biological membranes.

KEYWORDS: IAM Chromatography; log K_{IAMW}, intestinal permeability; blood brain barrier permeability.

INTRODUCTION

The therapeutic efficacy of an administered drug is dictated not only by its pharmacological properties such as potency and selectivity, but also to its pharmacokinetic properties such as its access to the site of action.[1] It has been found that failure of a drug candidate to reach the market happens very often during the development process because of inability of the candidate to cross biological membranes and reach desired site of action.[2] Hence, testing abilities of candidates to cross biological membranes is a crucial step in drug development. Several in-vitro approaches have been used for such purpose. These approaches include; physicochemical based models such as simple organic solvent/aqueous partitioning system, chromatographic partitioning, and partitioning into liposomes; cell assay based models such as caco-2 cells; and tissue assay based models, for example, inverted gut sac, and in situ intestinal perfusion.

Physicochemical based models have been found to be simple and non-invasive when compared to other models. Classic non-polar/polar solvents partitioning system, or simply shake-flask partitioning, has been successfully used for decades to determine lipophilicity of compounds. However, partitioning through such system correlates well with drug partitioning into fluid membranes for hydrophobic compounds; nonetheless, for polar compounds the correlations are not good.[3] On the other hand, partitioning into liposomes has been reported as a good system for predicting permeability of molecules over the other systems since they model both polar and non-polar solute-membrane binding interactions.[3] Permeability prediction of solutes has been extensively investigated on chromatographic systems as models that simulate solute partitioning in endogenous membranes.[4-6] Chromatographic partitioning has several advantages over other partitioning systems for its simplicity, reproducibility, and being qualitative rather than quantitative. It also needs small quantity of the solute and the use of high purity of the compound is not necessary.[4] Moreover, selectivity and accuracy of the model favor its use in solute partitioning. However, the use of octadecyl stationary (ODS) phase on silica support packed columns has not been found to ideally model the biological membrane due to its hydrophobicity surface.[3] For this reason, the system is only good for hydrophobic compounds.[3] To mimic internal membrane, Pidgeon and co-workers.[7] developed a phospholipid covalently

bonded to silica packed columns namely; immobilized artificial membranes. These columns have a monolayer of phospholipid molecules covalently bonded to the surface of silica particles.[8] The surface of the columns emulates the lipid surface and drug interaction as liposomes and cell membranes.[8-9] Therefore, the system is suitable for investigating non-polar and polar partitioning due to its structural similarity with biological membranes. Partitioning or binding of solutes to IAM columns is used to predict permeability based on retention time (t_r) parameter and the column void volume time (t_0) expressed as capacity factor, k'_{IAM}, as follows:[10,11]

$$k_{IAM} = [(t_r - t_0)/t_0] \quad (1)$$

Log k_{IAM} is used to evaluate lipophilicity of drug substances and predict their absorption.[10] Log k_{IAM} was found to significantly correlate with the log P[12], partitioning coefficients into liposomes[13], caco-2 cells permeability coefficient[10] permeability in skin tissues[14] and human absorption.[15] For a drug whose action is in the brain, investigation of the ability of the substance to cross the blood brain barrier is important. A common measure of BBB permeability is the ratio of the steady state concentrations of the drug molecule in the brain and in the blood, usually expressed as log BB.[16] Experimental determination of log BB is a difficult, expensive technique and the data is accompanied by high percentage of error.[17] Until now few studies[18-22] have been conducted to investigate whether IAM chromatography can be used to predict BBB permeability. A difficulty is associated with such studies due to limited availability of experimental log BB values. In this study, the capacity factors of a set of 21 compounds were determined and correlated with their reported log P and log BB values to determine whether molecular interaction on IAM columns can predict permeability of drugs through intestinal membrane and the blood brain barrier.

MATERIALS AND METHODS

MATERIALS

The following compounds were purchased from Sigma, Minnesota, USA: Chlorambucil, cimetidine, atenolol, acetaminophen, ibuprofen, ethanol, antipyrine, caffeine, carbamazepine, Physostigmine, thioridazine, pyrilamine imipramine, chlorpromazine, promazine, benzene, toluene, 1,1,1-trichloroethane, hexane and heptanes. Structure of the compounds is shown in "Fig. 1". TLR-I-04 was synthesized and characterized in our laboratory.[23] Acetonitrile (ACN) was purchased from Aldrich, Milwaukee, Wisconsin, USA.

METHODS

The IAM.PC.DD.2$^{C10/C3}$ column utilized (Regis Technologies, Inc., Illinois, USA) had the following specifications: 30 mm x 4.6 i.d., a 5 μm particle size diameter and a pore diameter of 300 Å. A liquid chromatograph equipped with UV detector (L-4000 UV, Hitachi Ltd, Japan) operating at varying wavelength according to the investigated compound was used. The mobile phase was water or mixture of water/ACN of different ratios pumped (L-6200A Intelligent Pump, Hitachi Ltd, Japan) at flow rate of 1 mL/min. Solution of each compound was prepared at ~3 x 10^{-4} M in water or water/ACN mixture and a sample of 5 μL was injected (AS-4000 Intelligent Autosampler, Hitachi Ltd, Japan). Data were recorded and integrated using Spectra-Physics Integrator (Model SP4270, Autolab Division, California, USA). The column was conditioned using 30 mL of the mobile phase and the system was equilibrated by making several injections of the compound until identical retention times were obtained. The dead time of the column was determined with acetone. Chlorambucil, cimetidine, atenolol, acetaminophen, ethanol, antipyrine, caffeine and physostigmine were eluted by completely aqueous mobile phase. All other compounds were eluted with mobile phase containing different percentages of the organic modifier. For compounds not eluted with aqueous mobile phase, log K_{IAM} was determined at different concentrations of ACN in the mobile phase. The resultant log K_{IAM} values were plotted vs. % of ACN and then extrapolated to 100% water mobile phase to yield log K_{IAMW} as shown in"Fig. 2".

RESULTS AND DISCUSSION

The partitioning of 21 structurally unrelated compounds set was studied on IAM column. The molecules vary in their chemical structure, physical properties, hydrophobicity (experimental log P ranges from -0.18 to 6.42) and pharmacological use. Log P, calculated and experimentally determined, and log BB values of the compounds obtained from literatures are shown in Table 1. The capacity factors of the compounds measured on IAM column were determined using aqueous mobile phase and in most cases isocratic organic modifier was used as co-solvent to elute the compounds. The organic modifier ACN in concentrations ranged 30-40% in the mobile phase was used. A plot of log K_{IAM} vs. % of ACN followed by linear extrapolating to zero percentage ACN was performed and presented in "Fig. 2". Profiles of some compounds were removed from the Figure for clarity purposes. For most of the compounds, linear relationships ($r \geq 0.985$) were obtained between the percentages of the organic modifier in the mobile phase and the retention times. However, the lowest correlation value was ($r = 0.954$).

Log K_{IAMW} correlation with log P

Lipophilicity, mostly expressed in log P, is one of the physicochemical factors widely used to predict membrane permeability after oral administration. Lipophilicity of drug molecule plays an important role in drug absorption, permeation and disposition.[4] The aqueous capacity factor of the compounds, log $K_{IAMW,}$ determined on IAM column and other physicochemical parameters are presented in Table 1. When log K_{IAMW} values of the compounds plotted against their experimental log P values, a correlation coefficient ($r = 0.892$) was obtained. Re-plotting of the data after

omitting three outliers, chlorambucil, thioridazine and TLR-I-04, the correlation was highly improved as shown in the following relationship and as can be seen in "Fig. 3". However, the separate effect of each outlier on the correlation is minor when it was incorporated alone within the set.

$\text{Log } K_{IAMW} = 0.559 \log P - 0.005$ (2)
n = 18 r = 0.952 s = 0.325

When log K_{IAMW} values of the compounds set were plotted against their calculated log P data, the respective correlation was slightly higher than that obtained with experimental log P (r = 0.905). After removing the outlier compounds, higher correlation was also obtained (r = 0.957). These results indicate the dependence of molecular partitioning into IAM surface on lipophilicity of the compound. The linearity between log P and log K_{IAMW} indicates the usefulness of the capacity factor to predict lipophilicity of drug molecules and hence their permeability. Other workers have reported similar correlations between aqueous capacity factors determined on IAM columns and log P.[10,24] Yang et al[10] found a better correlation (r = 0.985) between log P and log K_{IAMW} values for 15 phenethylamine derivatives. However, diversity of the compounds set in Yang's et al[10] study was not provided and their work dealt with structurally related chemical compounds.

Equation 2 was used to predict log K_{IAMW} values of the compounds. The predicted values were highly correlated with the experimental ones as shown in the following equation, "Fig. 4":

$\text{Log } K_{IAMW}$ (predicted) = 0.906 log K_{IAMW} + 0.117 (3)
n = 18 r = 0.952 s = 0.310

This high correlation suggests that IAM columns can be utilized for log P determination rather than using the classic shake-flask method or ODS chromatography. Although shake-flask method is a useful methodology, many technical difficulties are associated with the technique such as time and chemical consuming, pure solute and solvents should be used, possible instability of the solute in the solvent system and emulsion formation, which may hinder the separation and analysis. Thermodynamic partitioning of compounds on IAM surface also expresses the dynamic interactions between a flowing molecule and cell membrane rather than with shake-flask method which represents partitioning between two bulk phases.[25] IAM-HPLC is also preferred over ODS-HPLC where determination of log K_{IAM} is facile and easier rather than log P or the lipophilicity index (log K_w) determined on ODS column. Compounds of moderate lipophilicity can be eluted on IAM columns by aqueous mobile phase, which is time and cost effective.

Log K_{IAMW} correlation with log BB

A common measure of BBB permeability is the ratio of the steady state concentrations of the drug molecule in the brain and in the blood, usually expressed as log BB.[16] Log BB values of the compounds are obtained from literature and presented in Table 1. The determined log K_{IAMW} values of the compounds were plotted with their log BB data. TLR-I-04 was excluded from this analysis since its log BB was not available. The capacity factors of the compounds set showed an acceptable correlation with their log BB values (r = 0.601). When the date re-plotted after omitting chlorambucil and ibuprofen from the set, the respective correlation was greatly improved as can be seen in "Fig. 5" and expressed by the following equation:

$\text{Log BB} = 0.472 \log K_{IAMW} - 0.376$ (4)
n = 18 r = 0.767 s = 0.416

This correlation is higher than that was found by other workers[22] who reported a value of (r = 0.576) for a 29 compounds set. Many of those compounds in Salminen's and coworker's study[22] were used in this study. Equation 4 was used to predict log BB values of the compounds. A correlation value of (r = 0.587) was obtained when log BB data of 20 compounds plotted vs. predicted log BB. When 5 outliers were omitted (chlorambucil, ibuprofen, ethanol, carbamazepine and thioridazine) the linearity was greatly improved (r = 0.941) as seen in "Fig. 6". Taking into consideration that the compounds are of highly structural diversity and the set is not very large; these data indicate that the potential ability of IAM surface to emulate the BBB partitioning and the utility of log K_{IAMW} to predict BBB permeability. Using equation 4, a log BB value of 0.966 can be estimated for TLR-I-04.

Log BB correlation with log P

Many studies have directly related BBB permeability expressed as log BB with log P.[22,26] In our study log BB values of the compounds showed relatively lower correlations with calculated log P values (r = 0.518) and experimental log P values (r = 0.508). However, when log BB values were re-plotted vs. calculated log P and experimental log P data after omitting chlorambucil and ibuprofen, the correlations were improved. Correlations of (r = 0.763) and (r = 0.754) were found when log BB values were plotted vs. calculated log P and experimental log P values, respectively. In this study log K_{IAMW} has shown a slightly better correlation with log BB than did log P with respect to BBB permeability. Such better correlation is in accordance with other workers.[21] They have found better and high correlation of log K_{IAMW} vs. log brain uptake index (BUI) for six steroids and biogenic amines than those obtained for log P vs. log BUI.

Inclusion of molecular weight (MW) with log P data, log P/MW has been reported to improve the correlation with log BB. Levin has shown good correlation between BBB permeability with log P divided by the square root of MW, log $P/MW^{0.5}$.[27] A similar improvement has also

been reported when MW was included with log K_{IAMW} in a three parameter model and plotted vs. log BB.[22] In contrast, in this study, when log P/MW data of the set were re-plotted vs. log BB values, the correlation was reduced (r = 0.496). When chlorambucil and ibuprofen were removed, a correlation (r = 0.633) value was obtained. The same happened when log K_{IAMW}/MW and log BB values were re-analyzed (r = 0.648). When chlorambucil and ibuprofen log K_{IAMW} values were included in the plot, the correlation was greatly reduced (r = 0.491). Negative effects of the MW on the correlation of permeability data vs. molecular descriptors have also been reported.[28,29] Pannier and co-workers have found lower correlation of log P vs. % of skin permeability when log P data corrected with MW rather than using log P alone. Furthermore, negative coefficients for the molecular size in quantitative structure activity relationship equations that model brain distribution have been reported.[29] Though membrane penetrability depends on the molecular size of the penetrant; however, the effect of MW on the correlation values is still not clearly justified. This also raised a question by Kaliszan and Markuszewski[30] whether such improvement happens just by fortuitous artifact or can be rationalized. It is known that lipophilicity, the prime parameter that controls membranes permeability, is independent of MW to some extent. Salminen, et al[22] and his group have explained that large solutes favor partitioning into lipid and decrease the diffusion through membrane. Permeability is influenced by these two effects. Although such explanation is important, it cannot be easily applied. Combinatorial chemists have suited their efforts to obtain compounds of small MW and in the mean time lipophilic enough to cross the biological membrane. Lipinski and co-workers[31] have set a MW limit of 500 above which a compound is expected to be poorly permeable. Confining MW of compounds to below 500 is a hard task.

Drug permeability prediction potential of IAM chromatography

As can be seen in this study, log K_{IAMW} has been found to correlate well with parameters such as log P and log BB that are normally used to predict membranes permeability and in particular intestinal membrane and blood brain barrier, respectively. The IAM stationary surface has been reported to be better model for biological membranes than ODS's surface. This is in part due to not only hydrophobic but also hydrophilic compounds interacting with the IAM column's surface.[21] Such interactions are represented by good correlations of apparent partition value, log $D_{7.4}$ vs. log K_{IAMW}.[21,24] Good correlation (r = 0.800) has also been found when log K_{IAMW} of 10 compounds determined in this study was plotted vs. log capacity factors (log $K_{IAM7.4}$) of the same compounds eluted by phosphate buffer (pH 7.4) mobile phase determined by Salminen et al.[22] These compounds included cimetidine, acetaminophen, ibuprofen, antipyrine, caffeine, thioridazine, pyrilamine, imipramine, chlorpromazine and promazine. When ibuprofen was omitted from this group, the correlation was greatly improved (r = 0.923). The system has also shown good correlation with other parameters that can be used for evaluating permeability of drugs. Log K_{IAMW} showed good correlation (r = 0.791) with log % of intestinal absorption using perfused rat small intestine of 12 mostly acidic compounds than log P, which showed poor correlation (r = 0.10). Log capacity factors also gave better correlation with log of % absorption in mice (r = 0.941) than did log P (r = 0.890) for 11 cephalosporin compounds.[10] In our study, log K_{IAMW} of subset of 8 compounds (cimetidine, atenolol, acetaminophen, antipyrine, caffeine, carbamazepine, imipramine and chlorpromazine) has shown slightly better correlation with their log % of human absorption (r = 0.570) than did log P (r = 0.485). The usefulness of the IAM chromatography to predict membranes permeability of compounds is probably due to that the molecular partitioning process into IAM surface comprise hydrophilic, hydrophobic and also electrostatic interactions which are all involved in the process of partitioning of molecules into cell membranes (Kramer, et al., 1996).[32] Such multiple interaction characteristics of IAM surface increase the reliability of the data obtained from IAM partitioning and draw a conclusion that the system can be of value as in-vitro model for predictions of membranes penetrability.

Table 1: Physicochemical properties of the compounds.

Sr. No.	Compound	MW	Log K_{IAMW}	Exp. Log P^A	Calc. Log P^B	Log BB^C	Log $K_{IAMW7.4}{}^D$	% Human Absorption ^{E}
1	Acetaminophen	151.17	0.222	0.49	0.34	-0.31	0.185	80
2	Antipyrine	188.23	0.523	0.38	0.27	-0.10	-0.155	100
3	Atenolol	266.34	-0.124	0.14	0.22	-0.70		54
4	Benzene	78.11	1.143	2.13	2.22	0.37		
5	Caffeine	194.19	0.125	0.07	-0.07	-0.06	-0.229	99
6	Carbamazepine	236.27	1.645	2.67	2.67	0.00		100
7	Chlorambucil	304.21	1.067	3.70	3.70	-1.70		
8	Chlorpromazine	318.86	3.117	5.20	5.36	1.06	2.551	100
9	Cimetidine	252.34	0.358	0.21	0.36	-1.42	0.564	79
10	Ethanol	46.07	-0.777	-0.18	-0.19	-0.16		
11	Heptane	100.20	2.091	4.50	4.47	0.81		
12	Hexane	86.18	1.661	3.90	3.94	0.80		

13	Ibuprofen	206.28	2.519	3.68	3.72	-0.18	0.409	
14	Imipramine	280.41	2.207	4.41	4.47	0.83	1.818	99
15	Physostigmine	275.35	0.684	0.99	0.99	0.08		
16	Promazine	284.42	2.382	4.28	4.63	1.23	2.146	
17	Pyrilamine	285.38	2.101	2.77	3.26	0.49	1.213	
18	Thioridazine	370.58	2.416	6.42	6.13	0.24	3.055	
19	TLR-I-04	309.44	2.843	3.36	3.44	0.97		
20	Toluene	92.14	1.536	2.73	2.68	0.37		
21	1,1,1-Trichloroethane	133.40	1.327	2.49	2.10	0.40		

[A]Data of compounds 1,5,8,9,13,14,16-18 were taken from Salminen et al.,[22] 2-4, 10-12, 20, 21 from Kaliszan and Markuszewski,[31] 6,7,15 from Feher et al.,[33] and 19 from El-Gendy and Adejare.[23] [B]Data were taken from Feher et al.,[33] except the values of compound 3 and 19, which were calculated using Chemdraw software. [C]Log BB values were obtained from Feher et al.,[33] except for compound 3, which was calculated based on the published blood/brain ratio of the drug in Neil-Dwyer, et al.,[34] and for TLR-I-04, which was estimated. [D]Data were taken from Salminen, et al.[22] [F]Data of compound 1 was taken from Irvine et al.,[36] 3 and 9 from Stenberg et al.,[35] 6, 8, 14 from Wohnsland and Faller,[38] and 13 from Fredholm, et al. [37]

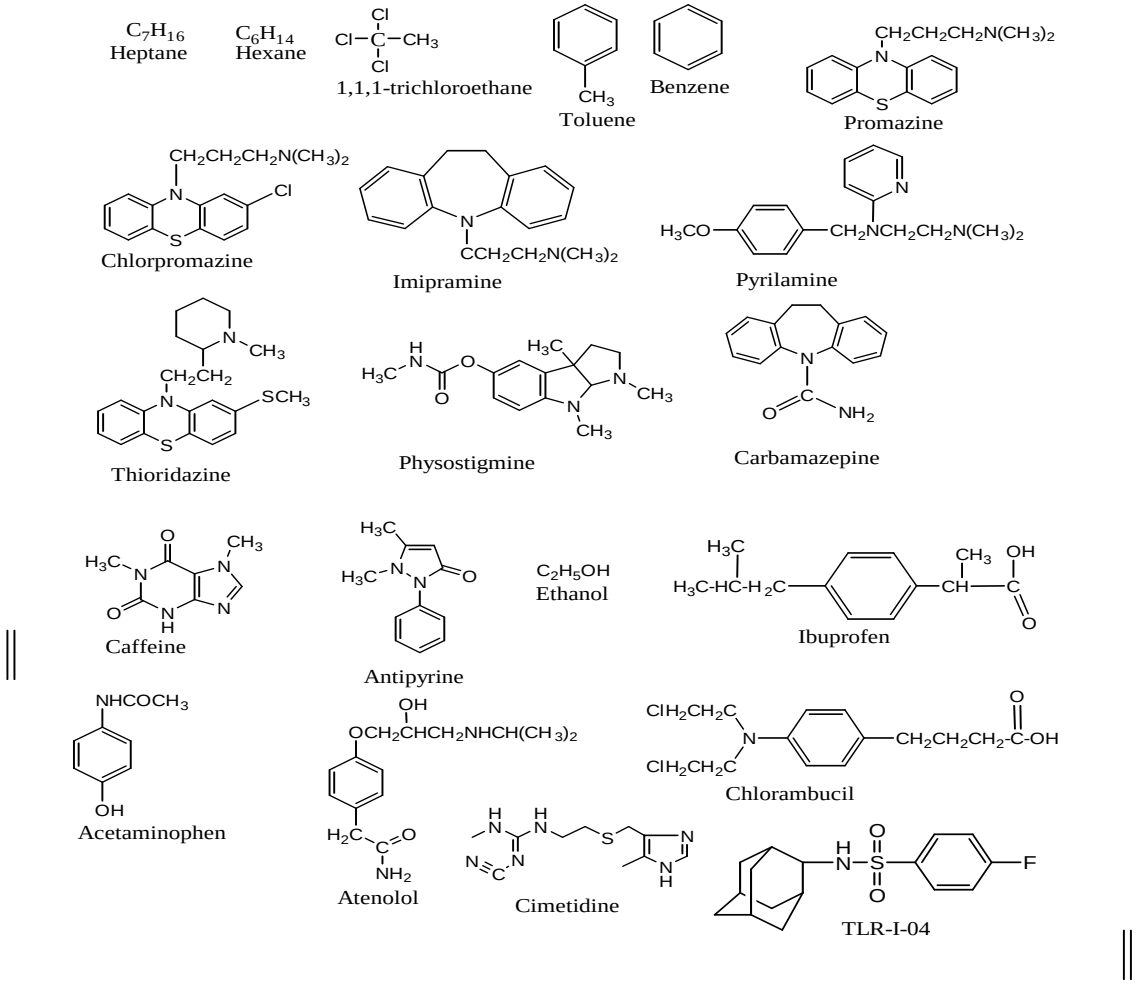

Figure 1. Structures of compounds utilized in the study.

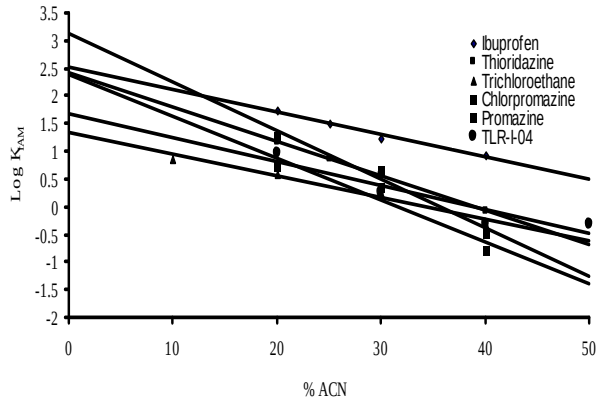

Figure 2: Relationship between % of ACN and log K_{IAM} of the compounds.

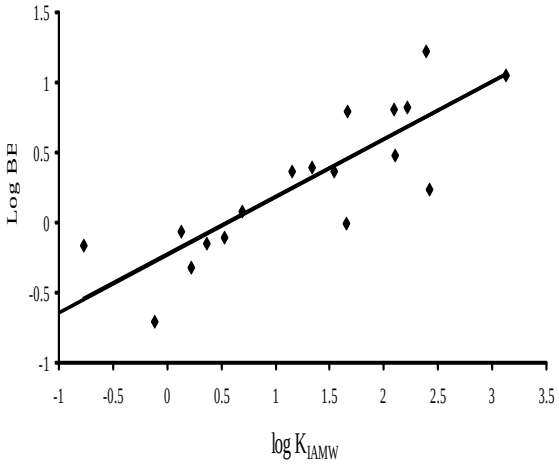

Figure 5: Correlation between log K_{IAMW} and log BB values of the compounds

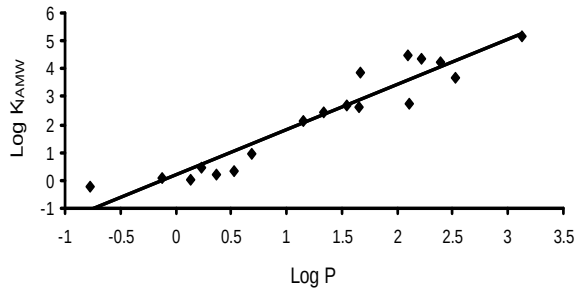

Figure 3: Relationship between log P and log K_{IAMW} values of the compounds

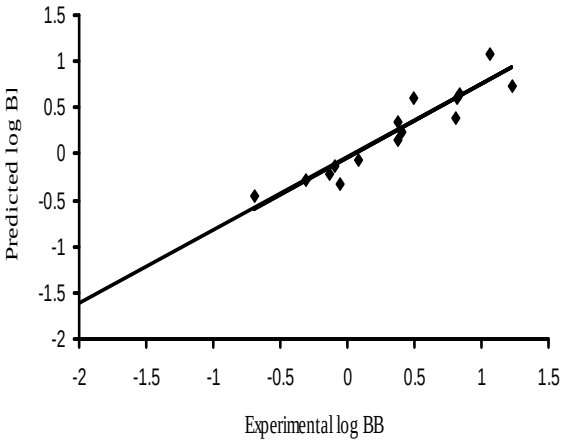

Figure 6: Relationship between experimental and predicted log BB values of the compounds.

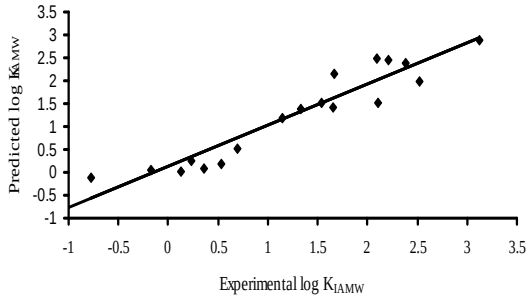

Figure 4: Correlation between experimental and predicted log K_{IAMW} values of the compounds.

CONCLUSIONS

The present study shows the usefulness of IAM chromatography to rank compounds regarding their ability to cross biological membranes. Log P of a compound reflects its ability to cross intestinal membrane. Good correlation of log K_{IAM} with log P values supports the screening potential of the capacity factor on IAM columns. Looking at the diversity and limitation of the compounds set used in the study, the blood brain barrier partitioning predictive ability of the system is promising. Due to ease of automation and high reproducibility, IAM-HPLC can be used as a high throughput screening tool for drug permeability through various biological membranes.

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