Predicting Drug Penetration Across the Blood-Brain Barrier: Comparison of Micellar Liquid Chromatography and Immobilized Artificial Membrane Liquid Chromatography

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INTRODUCTION

The Blood-Brain Barrier (BBB) permeability evaluation is an essential task for developing effective drugs for the treatment of the Central Nervous System (CNS). Both for drugs already on the market or under development, it is essential to know to what extent a drug enters the BBB. A common measure of the degree of BBB permeation is the ratio of the steady-state concentration of the drug molecule in the brain to the concentration in the blood, usually expressed as log ($C_{brain/blood}$) or log BB [1].

In this study, 45 compounds with available in vivo log BB values are analyzed with both Micellar Liquid Chromatography (MLC) and Immobilized Artificial Membrane (IAM) Liquid Chromatography. The capabilities towards log BB prediction are compared for both in vitro methods [2].

MLC is a mode of Reversed Phase Liquid Chromatography (RPLC) which uses a surfactant solution above the Critical Micellar Concentration (CMC) as mobile phase. The CMC phase allows for purely aqueous elution of most compounds within acceptable elution times on C_{18} columns. The drug interactions in MLC are presented in Figure 1A [3].

IAMs mimic the lipid environment of a cell membrane. They are prepared by linking phospholipid analogues to silica particles. This can be used as an HPLC column packing material (Figure 1B) [4].

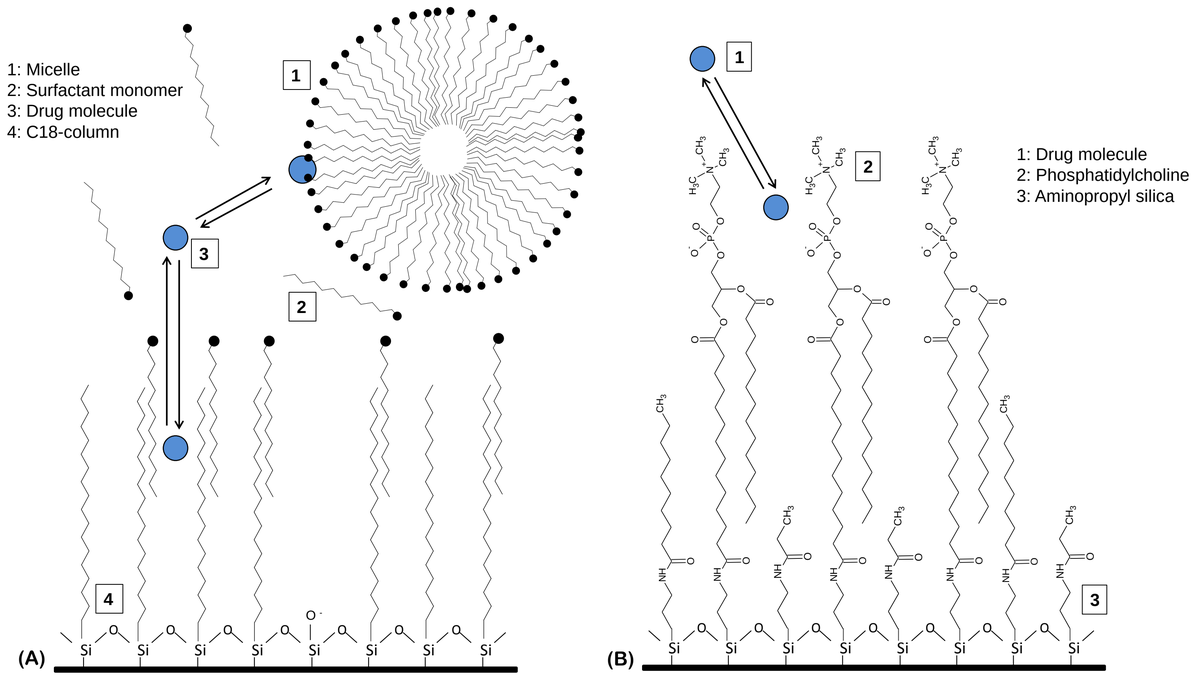

Figure 1: Schematic representation of drug interactions in Micellar Liquid Chromatography (MLC) (A) and in Immobilized Artificial Membrane (IAM) liquid chromatography (B)

EXPERIMENTAL

MLC

MLC measurements were performed on a GraceSmart C_{18} column (3 µm, 150 mm x 2.1 mm), the mobile phase flow rate was 0.2 ml/min. Three types of surfactants were used at a concentration of 0.05 M: Sodium Dodecyl Sulfate (SDS), polyoxy-ethylene (23) lauryl ether (Brij35) and Sodium DeoxyCholate (SDC). The surfactants were dissolved in a phosphate or borate buffer solution and the pH was set at 7.4.

IAM

IAM liquid chromatography measurements were performed on a Regis IAM.PC.DD2 column (10 µm, 150 mm x 4.6 mm), the mobile phase flow rate was 1 ml/min. The mobile phase was a mixture of methanol and Dulbecco's Phosphate-Buffered Saline (DPBS). Measurements were performed with 20, 30 or 40% methanol.

Log BB

The retention factors (k) of the compounds were measured. A Partial Least Squares (PLS) regression was performed in order to determine the correlation coefficient (R) between the experimental (in vivo) log BB values and log BB values predicted using log k values and several molecular descriptors. The most relevant descriptors were selected by systematic removal and/or reinsertion of all descriptors from the models while monitoring the effect on the Leave-One-Out Cross-Validation (LOOCV) regression coefficients.

RESULTS & DISCUSSION

The results from the PLS and LOOCV regressions are given in Table 1.

Table 1: Correlation coefficients between in vivo log BB values and predicted log BB values using log k values and several molecular descriptors

	Brij35 0.05 M	SDC 0.05 M	SDS 0.05 M	IAM 40 % MeOH	IAM 30 % MeOH	IAM 20 % MeOH	IAM 0 % MeOH	SDS + IAM 40 % MeOH	SDS + IAM 30 % MeOH
# compounds	43	36	45	45	45	39	45	45	45
R (PLS)	0.7870	0.8862	0.8564	0.8602	0.8659	0.8389	0.8621	0.8825	0.8848
R (LOOCV)	0.6620	0.7842	0.7993	0.7533	0.7724	0.7451	0.7831	0.7916	0.7982

The test set consisted of 45 compounds. Since an accurate prediction of log BB values for any type of drug is necessary, the conditions that allowed to measure all 45 compounds were considered most interesting (indicated in gray). Measurements with SDS as surfactant allowed for the best correlation, but results from the IAM column were comparable. Extrapolation of IAM results to 0 % MeOH hardly improved the correlation coefficient.

The correlation between in vivo and predicted log BB values is illustrated in Figure 2 for the 0.05 M SDS mobile phase before and after optimization. Although there are a few outsiders, the predicted log BB values for most compounds are close to the experimentally (in vivo) determined values.

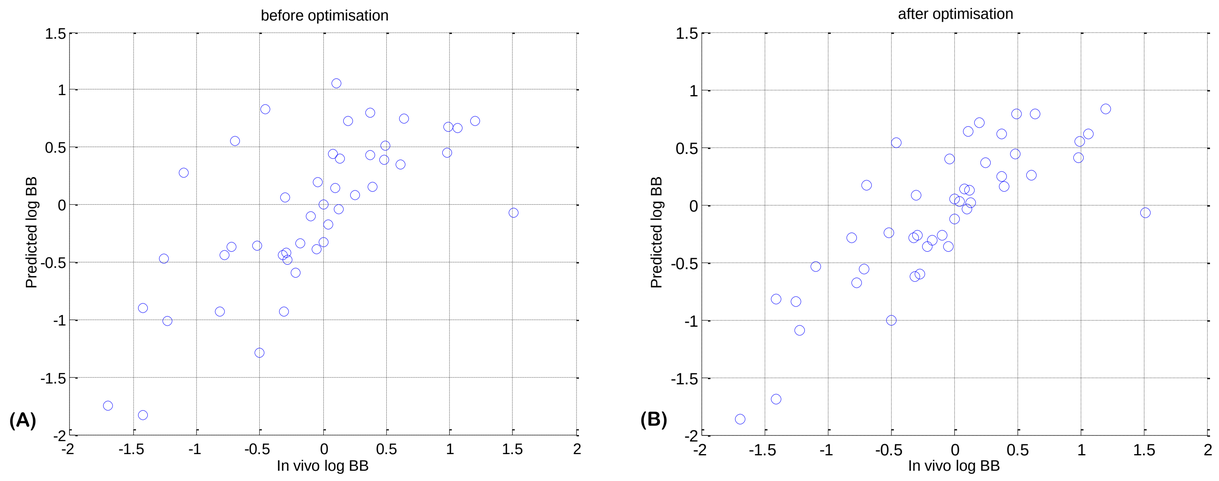

Figure 2: Visual representation of the correlation between 'In vivo' and 'Predicted' log BB values using the LOOCV method with log k values from 0.05 M SDS mobile phase on a C_{18} column before (A) and after (B) elimination of superfluous descriptors

Prediction of log BB values

The coefficients of the equations obtained from PLS regressions that lead to the R values listed in Table 1, are listed in Table 2. The general equation for Table 2 is: predicted log BB = a + b × α + c × Polarizability + d × log Wso + e × WS7.4 + f × PB + g × HIA + h × log k1 (+ i × log k2). Except for the log k values, all descriptor values are available in literature or can be calculated.

Table 2: Coefficients generated by PLS regression after elimination of several descriptors. The general equation for the predicted log BB values is:
log BB = a + b × α + c × Polarizability + d × log WSo + e × WS7.4 + f × PB + g × HIA + h × log k1 (+ i × log k2)

	Brij35 0.05 M	SDC 0.05 M	SDS 0.05 M	IAM 40 % MeOH	IAM 30 % MeOH	IAM 20 % MeOH	IAM 0 % MeOH	SDS + IAM 40 % MeOH	SDS + IAM 30 % MeOH
a	-3.666	-3.800	-3.911	-3.039	-2.995	-2.809	-2.859	-3.350	-3.302
b	0.589	0.241	0.397	0.455	0.495	0.437	0.600	0.324	0.358
c	-0.039	-0.053	-0.050	-0.044	-0.051	-0.053	-0.069	-0.046	-0.051
d	0.099	0.063	0.080	0.155	0.152	0.146	0.133	0.146	0.144
e	-0.002	-0.003	-0.003	-0.003	-0.002	-0.002	-0.002	-0.003	-0.003
f	0.007	0.007	0.011	0.003	0.002	0.003	0.004	0.005	0.005
g	0.044	0.048	0.044	0.047	0.046	0.042	0.042	0.045	0.045
h	0.530	0.439	0.571	0.709	0.705	0.517	0.604	0.453	0.466
i	-	-	-	-	-	-	-	0.344	0.323

CONCLUSION

- **SDS provides optimal log BB correlation by MLC on a C_{18}-column**
- **Comparable log BB correlation was obtained on an IAM column using a DPBS buffer with 30% MeOH**
- **The combination of methods with different interaction mechanisms does not lead to improved correlation coefficients**

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