

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



M. De Vrieze<sup>1</sup>, F. Lynen<sup>1</sup>, P. Sandra<sup>1,2</sup>

<sup>1</sup>Laboratory for Separation Sciences, Department of Organic Chemistry, Ghent University, Krijgslaan 281 S4-bis, B-9000 Ghent, Belgium

<sup>2</sup>Pfizer Analytical Research Centre, Ghent University, Krijgslaan 281 S4-bis, B-9000 Ghent, Belgium

## 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  $\log(C_{\text{brain/blood}})$  or  $\log \text{BB}$  [1].

In this study, 45 compounds with existing in vivo  $\log \text{BB}$  values are analyzed with both Micellar Liquid Chromatography (MLC) and Immobilized Artificial Membrane (IAM) Liquid Chromatography. The capabilities towards  $\log \text{BB}$  prediction are compared for both in vitro methods.

MLC is a mode of Reversed Phase Liquid Chromatography (RPLC) which uses a surfactant solution above the Critical Micellar Concentration (CMC) as mobile phase. MLC is a fascinating example of the benefits of secondary equilibrium in RPLC. The primary equilibrium is solute partitioning between bulk solvent and the stationary phase. A secondary equilibrium is established with the micelles in the mobile phase (Figure 1A)[2].

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)[3].

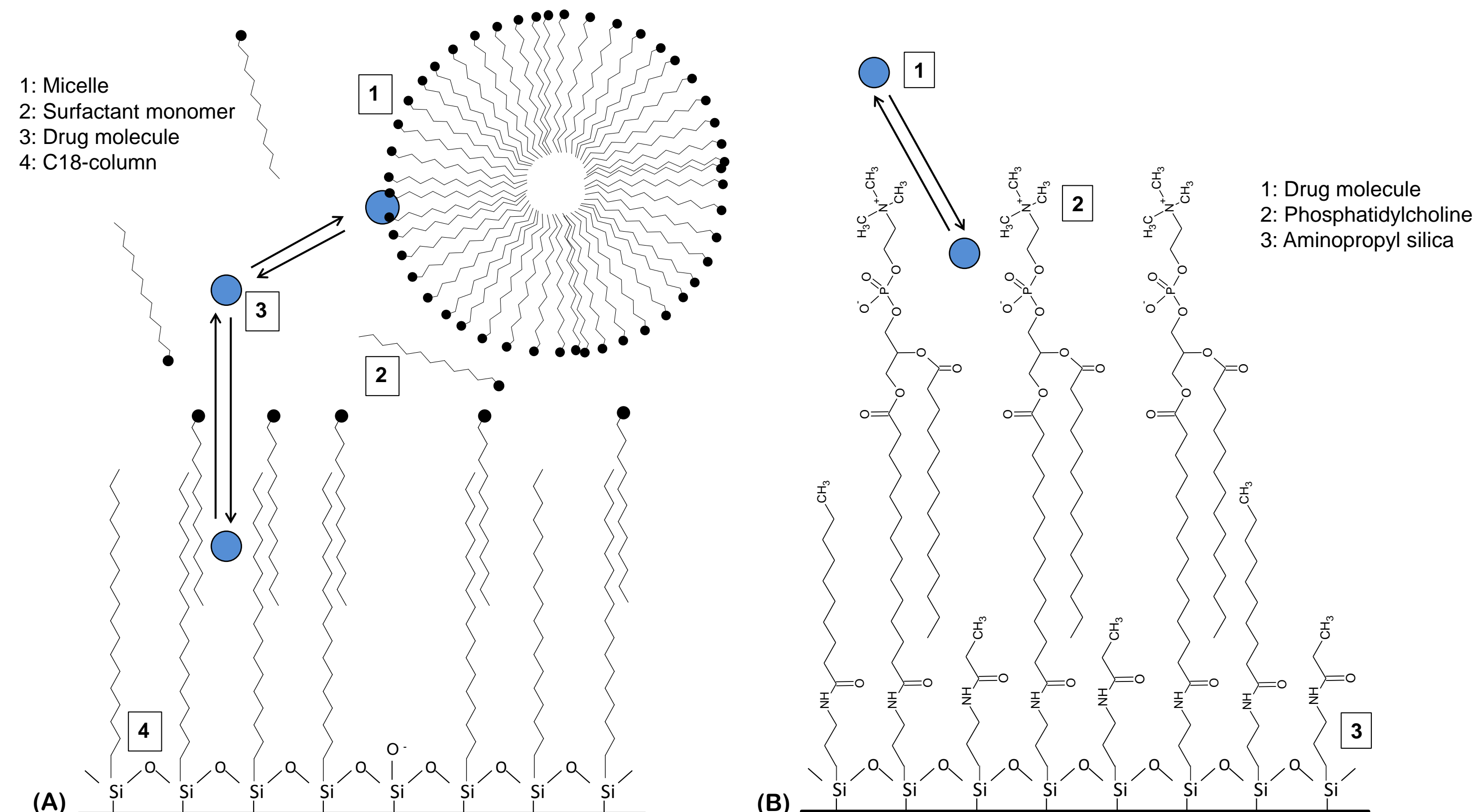


Figure 1: Schematic representation of drug interactions (A) in Micellar Liquid Chromatography (MLC) and (B) in Immobilized Artificial Membrane (IAM) liquid chromatography.

## EXPERIMENTAL

### MLC

In this study, MLC measurements were performed on a GraceSmart C18 column (3  $\mu\text{m}$ , 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  $\mu\text{m}$ , 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% of methanol.

### Log BB

The retention factors ( $k$ ) of the compounds were obtained using various mobile phases. A Partial Least Squares (PLS) regression was performed in order to determine the correlation coefficient ( $R^2$ ) between the experimental (in vivo)  $\log \text{BB}$  values and  $\log \text{BB}$  values predicted using  $\log k$  values and several other molecular descriptors.

## RESULTS & DISCUSSION

The results from the PLS regression are given in Table 1.

Table 1: Correlation coefficients between in vivo  $\log \text{BB}$  values and predicted  $\log \text{BB}$  values using  $\log k$  values and several molecular descriptors.

	Brij35 0.05 M	SDC 0.05 M	SDS 0.05 M	IAM 20% MeOH	IAM 30% MeOH	IAM 40% MeOH
# of compounds	43	36	45	39	45	45
$R^2$	0.8416	0.9213	0.8885	0.8659	0.8705	0.8689

The test set consisted of 45 compounds. Since the goal in this research is an accurate prediction of  $\log \text{BB}$  values for any type of drug, only the conditions that allowed to measure all 45 compounds were considered interesting (indicated in gray). Measurements with SDS as surfactant gave the best correlation coefficient, but results from the IAM column were also quite good.

The correlation between in vivo and predicted  $\log \text{BB}$  values is shown in Figure 2 for the two conditions with the highest correlation coefficient. Although there are a few outsiders, the predicted  $\log \text{BB}$  values for most compounds are very close to the experimentally (in vivo) determined values.

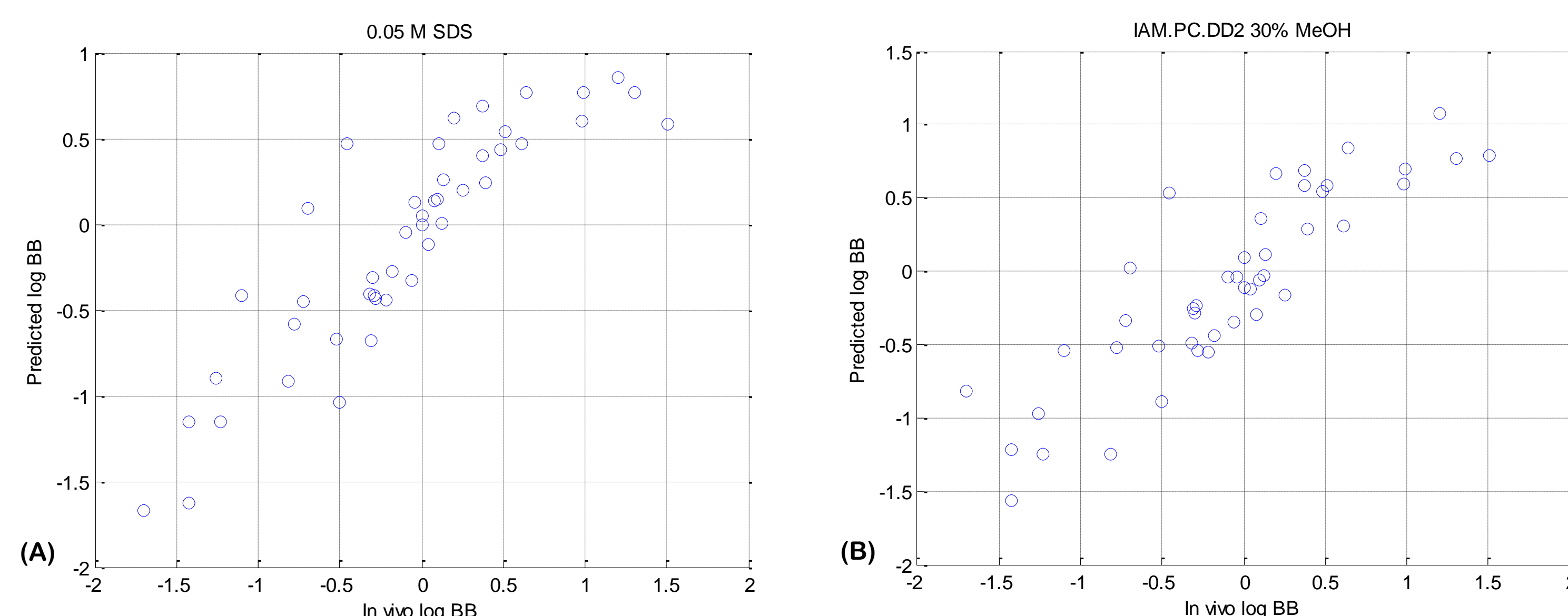


Figure 2: Visual representation of the correlation between 'In vivo' and 'Predicted'  $\log \text{BB}$  values (A) using SDS with MLC and (B) using 30% MeOH with IAM liquid chromatography.

### Combination of MLC and IAM

The selectivity for compounds is different when using a C<sub>18</sub>-column than when using an IAM-column (Figure 1). This has consequences towards the prediction of  $\log \text{BB}$  values. An extra PLS regression was performed in order to determine the correlation coefficient between in vivo  $\log \text{BB}$  values and predicted  $\log \text{BB}$  values using  $\log k_{\text{SDS}}$ ,  $\log k_{\text{IAM}}$  and several other molecular descriptors in the model. The obtained correlation coefficient was 0.8975 (Figure 3), which is almost 1% better than the correlation coefficient using only  $\log k_{\text{SDS}}$  values ( $R^2 = 0.8885$ ). The combination of these 2 types of  $\log k$  values thus leads to a significant improvement in the prediction of brain-blood concentration values of drugs.

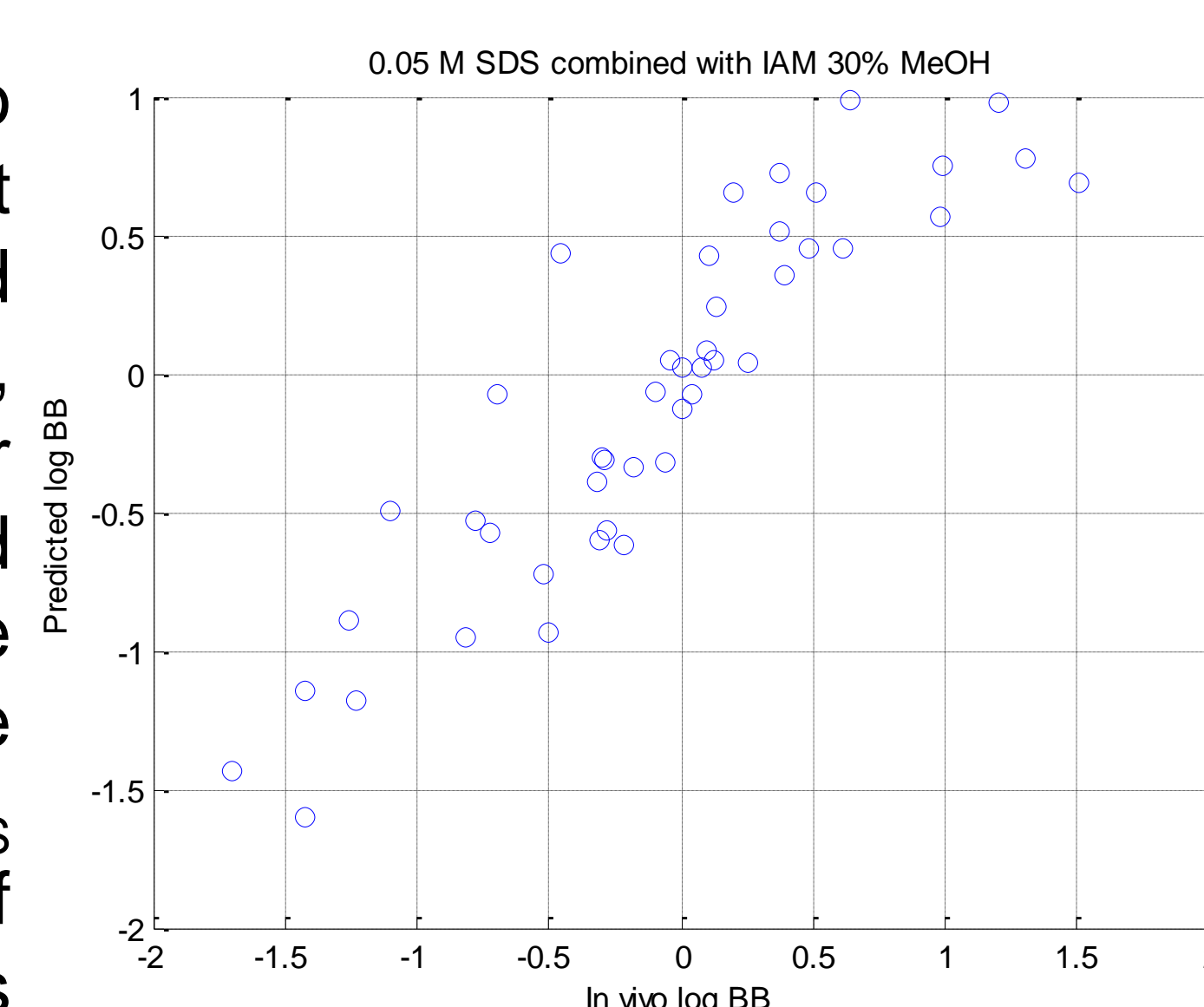


Figure 3: Visual representation of the correlation between 'In vivo' and 'Predicted'  $\log \text{BB}$  values when combining MLC and IAM methods.

## CONCLUSION

- ➔ SDS provides a good  $\log \text{BB}$  correlation on a C<sub>18</sub>-column
- ➔ 30% MeOH with a DPBS buffer gave a good  $\log \text{BB}$  correlation on an IAM column
- ➔ The combination of methods with different interaction mechanisms leads to a significant improvement in  $\log \text{BB}$  prediction.

## REFERENCES

- [1] L. Escuder-Gilabert, M. Molero-Monfort, R.M. Villanueva-Camañas, S. Sagrado, M.J. Medina-Hernández, J. Chromatogr. B 807 (2004) 193-201
- [2] M. Molero-Monfort, L. Escuder-Gilabert, R.M. Villanueva-Camañas, S. Sagrado, M.J. Medina-Hernández, J. Chromatogr. B 753 (2001) 225-236
- [3] S. Ong, H. Liu, C. Pidgeon, J. Chromatogr. A 728 (1996) 113-128