# *Very Thin PAMPA Membranes Indicate Higher Antipyrine Permeability but are Fragile and Contain Leaky Water Pores*

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## INTRODUCTION

The persistent difficulty of delivering therapeutic molecules across the blood-brain barrier (BBB) to achieve optimal CNS exposure continues to be a formidable challenge in the neuropharmaceutical industry. During drug discovery, costly *in vivo* measurements of brain penetration[1] are impractical, given the large number of molecules to test. This necessitates an ongoing search for simple and cost-effective models, such as PAMPA (parallel artificial membrane permeability assay), to predict the BBB permeation (*rate of brain penetration*) and other important properties relevant to successful CNS delivery (e.g., *extent of brain penetration)*.[1-3]

The first BBB application of PAMPA was described by Di *et al.*,[2] who used a 2%w/v porcine brain lipid extract (PBLE) in dodecane to coat filters to demonstrate that the PAMPA assay can successfully bin CNS+ and CNS- drugs. A new PBLE-based PAMPA model was recently reported,[3] using a five-fold higher PBLE lipid concentration in a more viscous alkane solvent than dodecane and with thinner membranes, compared to that used by Di *et al*. The newer BBB model matched the physicochemical selectivity of the rodent *in situ* brain perfusion permeability data precisely for acid and base drugs.

The purpose of the present study was to investigate the consequences of cutting back on the amount of PBLE lipid used to coat the PAMPA filters, to lower the cost of the assay and to increase the permeability values of test compounds, particularly that of antipyrine, a model test compound that has had lower than expected PAMPA permeability values in light of its high absorption classification. We report here the results of 1.0, 1.5, and 3.0 µL depositions of lipid-alkane mixtures on PVDF filters. The lowest volume was based on the use of pre-coated PAMPA plates purchased from BD Biosciences.[4] The two higher volumes were based on PBLE-coated filters.[3]

## PAMPA-BBB METHOD

The PAMPA Evolution Instrument (*p*ION) was used, employing magnetically-stirred STIRWELL™ 96-well plate "sandwiches." In the case of 1.5 and 3.0 µL depositions, the microfilters are automatically coated with a PBLE solution (*p*ION, PN 110672) by the robotic workstation. The Prisma™ universal buffer was used.

BD pre-coated PAMPA plates were purchased from BD Biosciences (Bedford, MA; PN 353015 – LOT 02059), based on 4%w/v DOPC in 1 µL hexadecane, as reported by the manufacturer.[4] The normal PAMPA 'sink' buffer could not be used with the BD plates due to their fragility, so a simple 10 mM HEPES pH 7.4 buffer was used in the receiver wells.

Data were analyzed according to the method described elsewhere.[3,5]

## RESULTS AND DISCUSSIONS

Figure 1a shows that octanol-water partition coefficients do not have the correct selectivity coefficient (slope in correlation plot),[5] SC, for modeling BBB permeability, and show high scatter for drug substances. Figure 1b shows the BD pre-coated model, with SC = 0.55. Figure 1c shows the PAMPA-BBB model for weak base drugs, based on PBLE lipid (3 µL/well), with near unit SC.

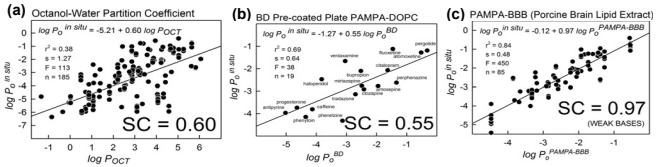


**Figure 1.** Selectivity coefficient plots for three model systems in relation to in situ brain perfusion intrinsic permeabiltiy values: (a) octanol-water partition coefficients; (b) BD pre-coated plates (PAMPA-DOPC); (c) PAMPA-BBB (3 µL/well 10%w/v PBLE in alkane), for base drugs.

Figure 2 shows as a function of gradient-pH the relationship between the PAMPA effective permeability coefficients, $P_e$ (solid), and the membrane coefficients, $P_m$ (dashed), with the upper dynamic range window (DRW) boundary formed by the aqueous boundary layer $P_{ABL}$ (dots), and the lower boundary by the paramembrane coefficients, $P_{para}$ (dash-dot), which describes the leakage of drugs through aqueous pores in the thin PAMPA membrane. The left frames describe the less leaky system based on 3 µL PBLE lipid deposit, and the right frames describe the very leaky system based on 1 µL DOPC lipid BD pre-coated plates.

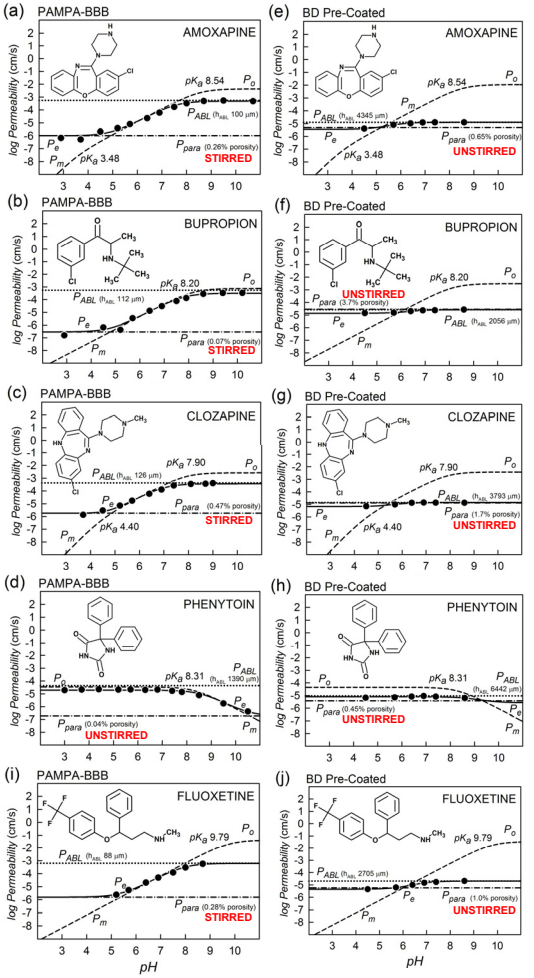


**Figure 2.** Left: large dynamic range window (DRW) of PAMPA-BBB based on 3 µL/well 10%w/v PBLE in alkane. Right: small DRW of BD pre-coated plates based on 1 µL/well 4%w/v DOPC in dodecane, due to excessive compound leakage (0.5-3.7% porosity) via aqueous pores.

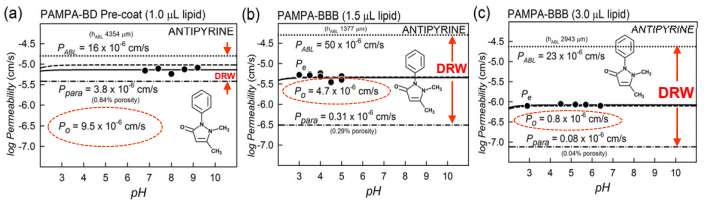


**Figure 3.** The intrinsic permeability of antipyrine, $P_o$, depends on the thickness of the PAMPA lipid barrier. The BD pre-coated plates have the thinnest lipid barriers (1.0 µL) and have the highest $P_o$ (9.5 x $10^{-6}$ cm/s). However, with thin membranes, the paramembrane is leakiest, which dramatically reduces the dynamic range window (DRW). The thickest PAMPA membranes (3.0 µL lipid) have the lowest $P_o$ (0.8 x $10^{-6}$ cm/s), but have the largest DRW.

Antipyrine, a high-permeability designated drug, is thought to be absorbed intestinally by passive transcellular diffusion. However, its PAMPA value is not as high as expected from its absorption classification. The Garberg *et al.*[6] results for antipyrine showed a basolateral/apical (BA/AB) efflux ratio of 0.8, possibly hinting of an unrecognized carrier-mediated uptake process. We are not aware of other studies suggesting a non-passive mechanism of antipyrine uptake.

Chen *et al.*[4] hypothesized a lipid/oil/lipid tri-layer structure for the BD pre-coated filter barriers. Since the void volume in the PVDF filter is calculated to be about 2.6 µL/well, 1 µL lipid volume used in the pre-coated plates is not enough to fully plug the filter inner volume. As a result, there may have been some water channels formed under the gradient-pH conditions.

## CONCLUSIONS

The new PAMPA-BBB model (3 µL/well 10%w/v PBLE in alkane) can precisely mimic the physicochemical microenvironment of the BBB governing passive permeability of ionizable drugs, with selectivity constants 0.97 for bases (Fig. 1c) and 1.08 for acids,[3] using the rodent *in situ* brain perfusion data as a benchmark. The *in combo* PAMPA-BBB technique improved the general performance for all classes of compounds, using 197-drug training set *in situ* "efflux-inhibited" rodent brain perfusion data ($r^2$ = 0.93; cross-validated $q^2$ = 0.92±0.03).[3] It was discovered that very thin PAMPA lipid barriers possess water channels that facilitate paramembrane aqueous diffusion of compounds. This was an extensive shunting effect, possibly limiting the determination of low-permeable compounds and obscuring pH-dependence of permeability with ionizable compounds. The 3 µL-coated PAMPA-BBB filters were most robust and had the largest dynamic range window, DRW. We have thus developed a practical, low-cost, and fast quantitative method which could be used for early passive BBB permeability screening, and for assisting medicinal chemists with structure modification to improve the BBB permeability of test compounds downstream in the CNS drug discovery process.

## ACKNOWLEDGEMENTS

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