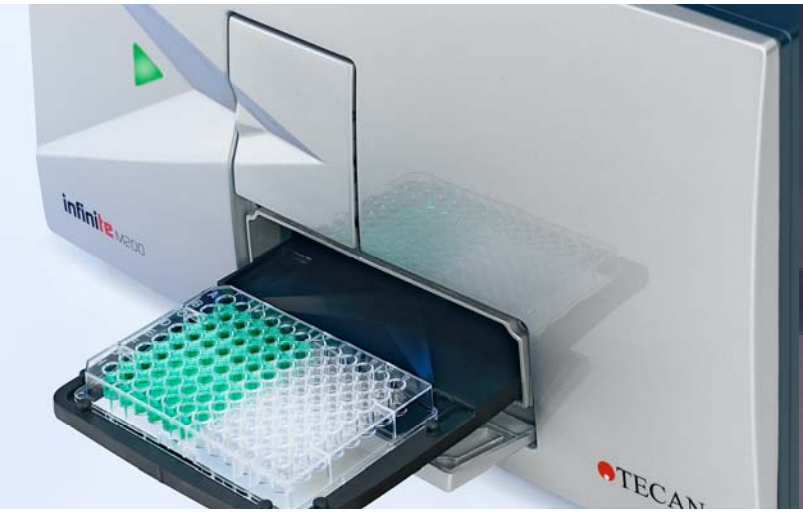
Application Note

Double-Sink™ PAMPA Assay for Permeability Studies

Implementation on Tecan´s Infinite® 200 Multimode Reader



Introduction

In vivo kinetic studies of drug uptake across the gastro-intestinal tract (GIT) and blood-brain barrier (BBB) are valuable tools for assessing bioavailability to prospective targets. These are relatively expensive and time consuming assays which are conducted sparingly.

*p*ION Inc. has introduced a parallel artificial membrane permeability assay (PAMPA) which has recently gained popularity as a novel, cost-effective high-throughput assay capable of rapidly screening compounds for their permeability characteristics in early drug discovery.
The method, called Double-Sink PAMPA, uses an optimized mixture of phospholipids infused into lipophilic microfilters, with net negative lipid charge, which mimics the different lipid composed membrane lipids of the GIT or BBB.

Analytical techniques for sample or compound detection are e.g. HPLC-UV or LC/MS, with the disadvantages of being cumbersome and expensive.
In this note we describe the easy implementation of Tecan´s Infinite M200 with its absorbance scanning feature for PAMPA sample analysis.

Assay Principle

The PAMPA assay for permeability measurements is based on diffusion of a compound of interest through an artificial membranes
A two compartment 96-well microtiter plate “sandwich” divide a donor and receiver compartments for the permeability assay (Fig. 1). Microfilters are coated with a proprietary phospholipid-based mixture. One lipid mixture and test conditions is specifically designed for the GIT; another is designed for the BBB.

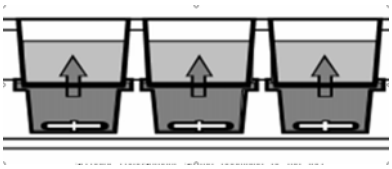


Figure 1. The PAMPA sandwich contains a lower donor compartment containing drug and an upper drug-free acceptor compartment at the beginning of the test. Magnetic stirrers in the donor control of the aqueous boundary layer.

The receiver compartment contains a specially developed, proprietary buffer which contains a chemical scavenger simulating the sink or binding conditions of *in vivo* systems.

After permeation and individual well stirring with the *p*ION Gut-Box™, the sandwich is disassembled and the permeability coefficients are calculated from the relative sample concentrations in the donor and acceptor wells, with correction for mass balance.

Materials and Methods

Instruments

- Tecan Infinite M200 multimode microplate reader
- PAMPA™ Explorer (*p*ION Inc, MA)
- Gut-Box™ (*p*ION Inc, MA)

Measurements

This system uses the Tecan´s multimode reader Infinite M200 with its UV-VIS absorbance scanning mode.
The PAMPA Explorer software collects and displays the data so the operator can confidently interpret the color coded results.

Measurement Parameters	Instrument Settings
Plate definition	GRE96ft_half area
Mode	Absorbance Scan
Start wavelength	230 nm
End wavelength	500 nm
Step	4 nm
Number of Reads per well	3

Results

Gastrointestinal Tract (GIT) Validation

A training set comprised of *in situ* human jejunal permeability data for 8 compounds was used. More than 70 lipid models were tested in the selection of the best test conditions for the Double-Sink PAMPA assay (see Fig. 2).

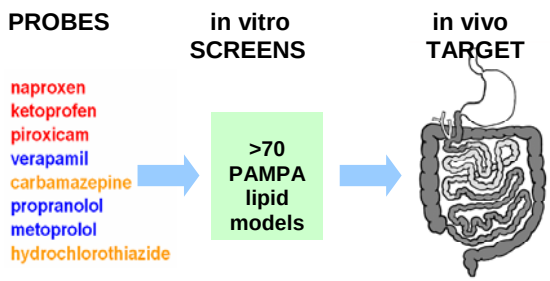


Figure 2. Schematic of GIT development

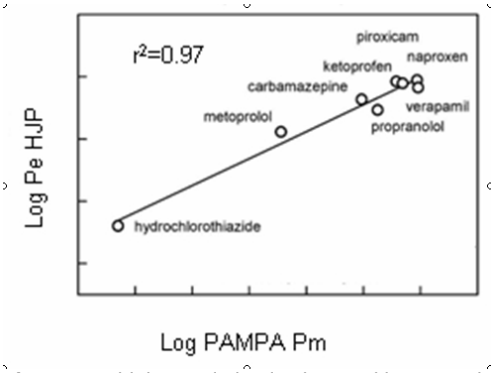


Figure 3. A high correlation is observed between the human jejunum and Double-Sink PAMPA.

Blood Brain Barrier (BBB) Validation

A training set comprised of *in situ* rat brain perfusion data for 130 compounds was used. More than 20 models were tested in the selection of the best test conditions for the Double-Sink PAMPA-BBB assay (see Fig. 4).

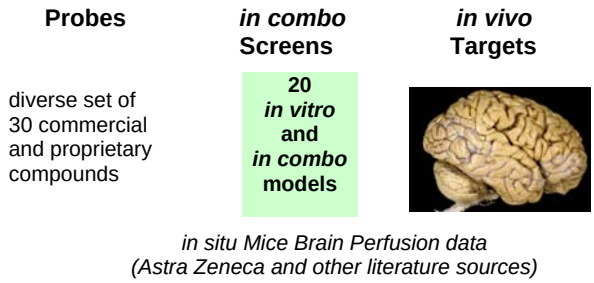


Figure 4. Schematic of BBB development

The BBB lipid, Brain Sink Buffer (BSB) compositions and conditions (incubation time, stirring, pH, etc.) of the PAMPA assay were optimized as shown in Figure 4.
The BBB PAMPA model was further evaluated to find the best single measurement that predicts the log PS value. Figure 5 shows the results where the rat brain perfusion data is highly correlated with the Double-Sink PAMPA-BBB results under Gut-Box stirring conditions which maintains the ABL thickness < 100 µm. The r^2 value reaches 0.92 with the aid of in-combo.

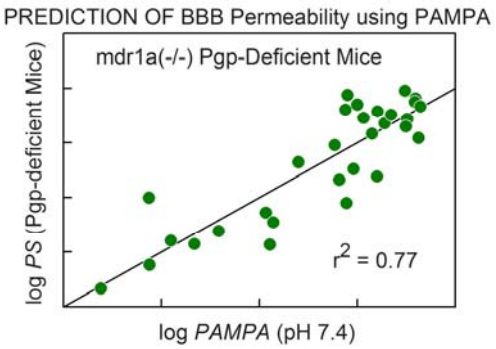


Figure 5. Log of the apparent BBB permeability vs. the log PAMPA values, measured at pH 7.4.

Conclusion

The results of the research demonstrate that it is possible to predict passive *in situ* permeation in the human jejunum and blood brain barrier using the PAMPA Explorer system in combination with Tecan´s Infinite M200 multimode microplate reader.
This approach has a higher throughput and is less expensive than *in vivo* experimentation, which enables more efficient upstream screening of compounds. Later, after additional work is completed, the compounds can be subjected to more traditional tests as required.

The PAMPA Explorer system implemented on Tecan´s Infinite M200 multimode microplate reader comprises a quick, reliable system that accurately models both human jejunal permeability (GIT) and rodent blood-brain barrier (BBB) uptake of candidate drug molecules, based on measurements of permeability across artificial phospholipid membranes.
This system can easily be adopted for high throughput on Tecan´s Freedom EVO series versatile liquid handling platform and flexible robotic workstation.

Abbreviations

BBB	blood-brain barrier
GIT	gastrointestinal tract
PAMPA	Parallel Artificial Membrane Permeability Analysis

Acknowledgements

We express our acknowledgements to Konstantin Tsinman and his group for performing the experiments and providing their data for this application note.
BBB research was made possible by National Institutes of Health (US) SBIR Phase I Grant (2005-2006) & special collaboration with AstraZeneca, Wilmington.

Austria +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 10 5869 5936 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Portugal** +351 21 000 82 16 **Singapore** +65 644 41 886
Spain +34 93 490 01 74 **Sweden** +46 31 75 44 000 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200
Other countries +41 44 922 8125

www.tecan.com

395 789 V1.0, 02-2009