Low Cost Solution for PAMPA Assays

Using the Epoch™ Microplate Spectrophotometer with *p*ION's PAMPA Explorer™ for the In-vitro Drug Permeability Studies

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A low cost monochromator-based absorbance microplate reader was used to assess drug permeability using *p*ION's patented Double-Sink™ method of PAMPA assays. The low background noise available with the microplate reader enabled the sensitive measurement of passive transport of small molecules across the artificial membrane. This provides a complete solution for low cost PAMPA assays.

Introduction

Drug permeability and solubility are for the most part offsetting factors that to a large extent govern the bioavailability of orally administered small molecule drugs (Figure 1).

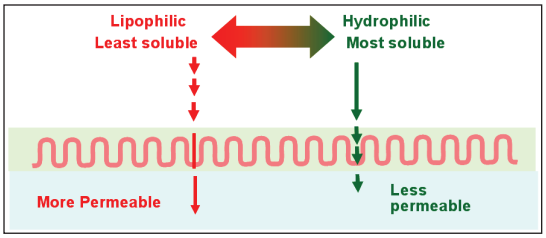

Figure 1. Relationship between solubility and permeability: high solubility ensures dissolution in the aqueous environment of the gut, but hinders passage across lipophilic membranes like the gastrointestinal lining; low solubility hinders dissolution, but allows compounds pass more freely across the membrane.

This is an important consideration in the optimization of confirmed hits originating from screening programs and entering ADME/Tox evaluation. PAMPA (parallel artificial membrane permeability assay) is a method which determines the passive permeability of lead compounds through an artificial membrane in a microplate in-vitro format that is a model for gastrointestinal in-vivo absorption. The in-vitro artificial membrane format allows for the rapid screening of compounds early in the drug discovery process (Figure 2). The Double-Sink™ method available from *p*ION in its PAMPA Explorer™ kits has industrialized the PAMPA method such that reproducible, accurate results can be obtained by users unfamiliar with the technique. The Double-Sink™ model uses pH gradient and an acceptor sink to mimic in vivo passive transport. This method has been shown to correlate well with human absorption [1-3].

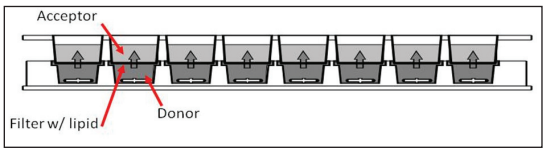

Figure 2. Cross-section of PAMPA Explorer™ sandwich plate: compound is loaded into Donor compartment and allowed to passively (with stirring) pass through the filter with lipid (artificial membrane) over a defined time period. Permeability is determined by measuring the absorbance of compound in the Donor and Acceptor compartment and comparing them to the initial compound's absorbance.

In this application note we will demonstrate the attributes of a low cost monochromator-based absorbance microplate reader that provide a complete solution for performing manual PAMPA assays.

Materials and Methods

PAMPA

PAMPA Explorer™ kits used for the evaluation of the microplate reader were obtained from *p*ION INC. Each kit includes 10 PAMPA sandwich microplates, PAMPA Explorer Command Software, Prisma™ HT universal buffer concentrate, GIT-0 lipid solution, acceptor sink buffer (ASB) and all necessary consumables including UV transparent microplates. All assays were performed to manufacturer's specifications. The Gut-Box™ was used during permeability assays to stir solutions in the Donor compartment. Stirring is calibrated to specific speeds so that the PAMPA assay mimics the expected thickness of the aqueous boundary layer in the gastrointestinal tract.

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PAMPA Measurements with Epoch™ Microplate Spectrophometer

PAMPA assays require absorbance spectra to be acquired in the UV-Vis range because the absorbance properties of research compounds are unknown a priori. Typically, small molecule compounds have UV chromophores such as double bonds between carbon atoms or phenyl rings that can be utilized. PAMPA spectra are typically acquired from 250 – 500 nm using the scanning monochromator in the Epoch Microplate Spectrophotometer.

Figure 3. Epoch Microplate Spectrophotometer: a low cost monochromator-based absorbance microplate reader.

Several UV-Vis measurements are made in PAMPA assays. First, a reference sample of the compound to be analyzed is made at a relatively high concentration in Prisma HT buffer (i.e. 50 - 100 µM) and transferred to the UV plate for measurement. The same solution is then added to the Donor compartment of the PAMPA sandwich. ASB buffer is added to the Acceptor compartment. The PAMPA sandwich plate is then placed on the Gut-Box, typically for 30 minutes incubation. Following this, both Donor and Acceptor samples are transferred to the UV plate and their spectra analyzed. Permeability coefficients of the compounds are determined from these measurements [4] through the use of the PAMPA Explorer software.

Results and Discussion

Background Signal in Acceptor Compartment Measurements

Due to the fact that a portion of the compounds tested may exhibit low permeability, and therefore low absorbance values, the background absorbance signal measured from the Acceptor compartment needs to be below 10^{-3} OD. The technique utilizes natural chromophores in the compounds being tested, so low background signals and noise are mandatory for accurate measurements of permeability. Figure 4 demonstrates the background signal from absorbance measurements made by the Epoch Microplate Spectrophotometer in the UV/Vis range from 250 – 500 nm using buffer as a sample. The average noise level is 0.0003 OD and does not exceed 0.0005 OD across the spectrum measured. This low background is satisfactory for accurate measurements of drug permeability.

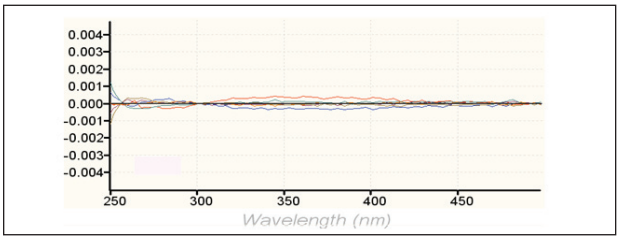

Figure 4. Absorbance measured in optical density units from 250 – 500 nm using the scanning monochromator of the Epoch Microplate Spectrophotometer.

PAMPA Measurements with Metoprolol

Figure 5 is characteristic data presentation from PAMPA Explorer software which demonstrates the ability to quantify Acceptor compartment solution concentrations of metoprolol as low as 1 µM. This ability is a direct result of the low background signal and noise level at the peak absorbance of the compound in the UV.

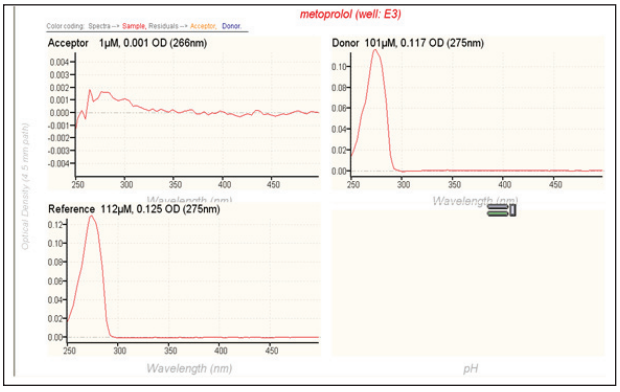

Figure 5. Detailed view of PAMPA Explorer software measuring absorbance spectra from metoprolol in Donor compartment, as a reference solution and from the Acceptor compartment demonstrating the permeability of the compound through the PAMPA membrane.

Conclusions

We have demonstrated the ability of Epoch Microplate Spectrophotometer to perform PAMPA assays with satisfactory signal to background. The example of metoprolol demonstrates an ability to determine permeability coefficients using PAMPA Explorer within 30 minutes. The Epoch is a low cost monochromator-based absorbance reader which will substantially reduce the capital cost of enabling Double-Sink PAMPA assays in laboratories.

References

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