

Automation of Pre-coated PAMPA Plates Improves Predictability, Reproducibility, and Efficiency

Xiaoxi (Kevin) Chen, Anthony Murawski, Michael Shanler,
Linda Hladik, and Charles L. Crespi

BD Biosciences - Discovery Labware, Two Oak Park, Bedford, MA 01730

Application Note

Contents

- 1 Introduction
- 2 Materials and Methods
- 3 Results
- 7 Conclusions
- 7 References

Introduction

Parallel Artificial Membrane Permeability Assay (PAMPA) is a useful screening tool for ADME properties (membrane permeability). A good PAMPA method is expected to be easy to use, automatable, and validated with a set of known drugs which have human absorption data.

In the traditional PAMPA method (*References 1-3*), PAMPA users need to prepare the artificial membrane themselves by coating the filter plate with a solution of lipids because the traditional artificial membrane, once prepared, is not stable for long-term storage. Another challenge of the traditional PAMPA method is the predictability, judged from how well the PAMPA results correlate with the human absorption data of known drugs. While the traditional PAMPA method provides good predictability for many compounds, it is challenged by the incorrect prediction of a group of drugs that are classified by the biopharmaceutical classification system (BCS) as high permeability compounds.

To improve the PAMPA model, we have developed a stable artificial membrane that can be pre-coated and stored for later usage. We have designed the artificial membrane to better mimic the biological membrane. The pre-coated PAMPA plates (BD Gentest™ Pre-coated PAMPA Plate System) have been validated for automation compatibility, stability, reproducibility, and predictability using a set of known drugs with human absorption data.



Materials and Methods

The BD Gentest™ Pre-coated PAMPA Plate System (Cat. No. 353015) was used to perform permeability assays for a set of commercially available drug compounds. The permeability assay was carried out using a Tecan® Genesis 150 liquid handler that is integrated to a plate reader using a robot arm. The assay protocol is the same as described in Reference 4. In summary, the 96-well filter plate, pre-coated with lipids, was used as the permeation acceptor and a matching 96-well receiver plate was used as the permeation donor. Compound solutions were prepared by diluting 10 mM DMSO stock solutions in PBS (in most cases we used a final concentration of 200 μM). The compound solutions were added to the wells (300 μL/well) of the receiver plate and PBS was added to the wells (200 μL/well) of the pre-coated filter plate. The filter plate was then coupled with the receiver plate and the plate assembly was incubated at room temperature without agitation for five hours. At the end of the incubation, the plates were separated and 150 μL solution from each well of both the filter plate and the receiver plate was transferred to UV-transparent plates. The final concentrations of compounds in both donor wells and acceptor wells were analyzed by a UV plate reader. Permeability of the compounds were calculated using the following formula:

$$\text{Permeability (cm/s): } P_e = \{-\ln[1-C_A(t)/C_{eq}]\}/[A*(1/V_D+1/V_A)*t],$$

where A = filter area (0.3 cm²), V_D = donor well volume (0.3 mL), V_A = acceptor well volume (0.2 mL), t = incubation time (seconds), C_A(t) = compound concentration in acceptor well at time t, C_D(t) = compound concentration in donor well at time t, and C_{eq} = [C_D(t)*V_D+C_A(t)*V_A]/(V_D+V_A).

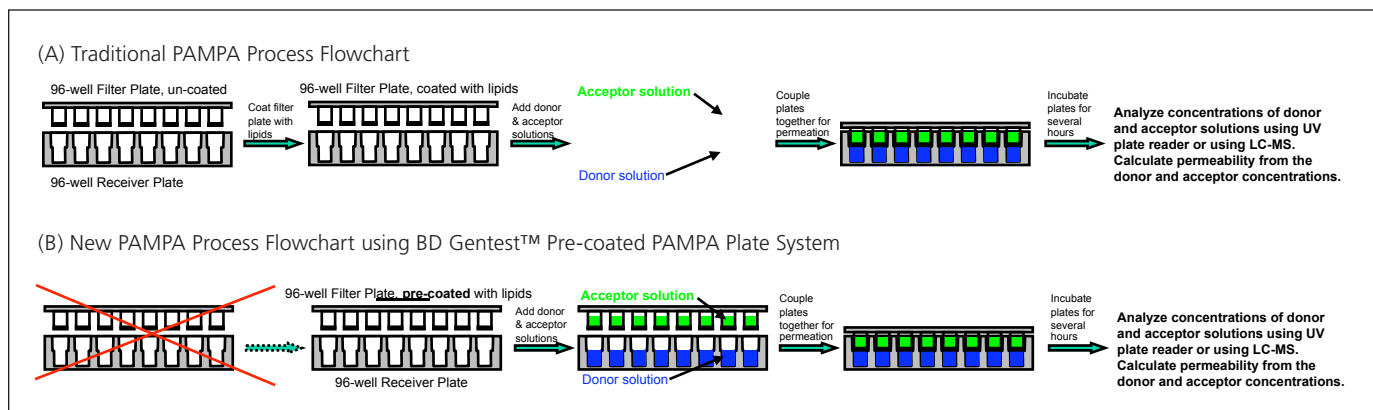


Figure 1. Experimental Setup of PAMPA.

- In the traditional PAMPA method, the user needs to prepare the artificial membrane by coating the filter plate with a lipids solution
- In the new PAMPA method, the BD Gentest™ Pre-coated PAMPA Plate System is ready to use, allowing the user to skip the step of preparing the artificial membrane

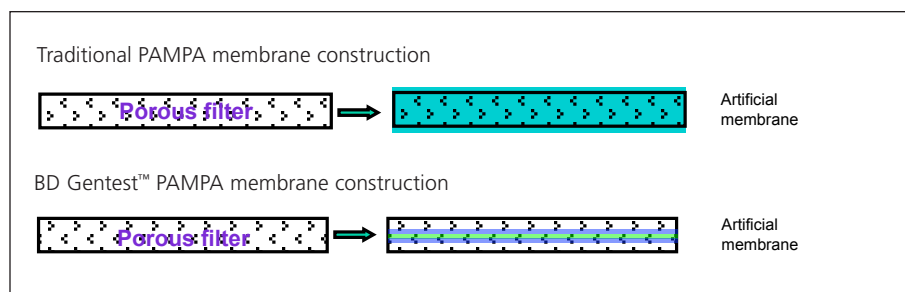


Figure 2. Comparison of the Traditional PAMPA Membrane and the BD Gentest™ PAMPA Membrane.

Traditional PAMPA membrane construction:

- Coated by user (by adding a solution of lipids in each well)
- The porous filter is soaked with a solution of lipids
- Excessive solvents
- Long permeation pathway

BD Gentest™ PAMPA membrane construction:

- Pre-coated by manufacturer
- A lipid-oil-lipid tri-layer structure is constructed in the pores of the porous filter
- No excessive solvents
- Short permeation pathway (closer to the biological membrane)

Presented as a Poster, Society for
Biomolecular Sciences Meeting 2008

Automation of Pre-coated PAMPA Plates Improves Predictability, Reproducibility, and Efficiency

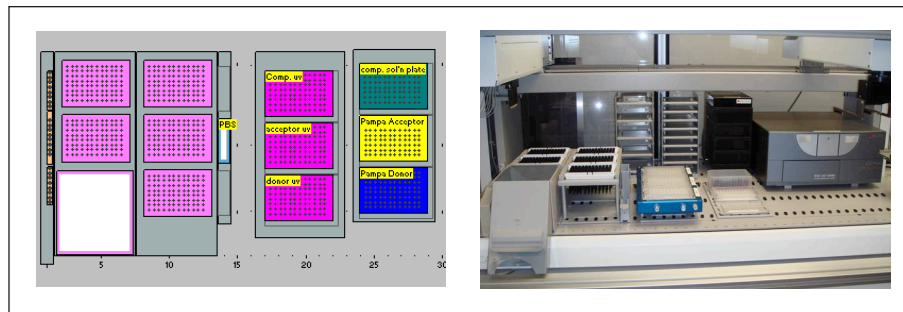


Figure 3. Automated PAMPA Deck Layout on a Tecan® Genesis 150 Configured with an Integrated Robotic Arm, UV Plate Reader, and Incubator.

A standard Tecan® Genesis 150 robot deck had the capacity to fit all the necessary consumables to run the assay: one (1) BD Gentest™ Pre-coated PAMPA Plate System (BD cat. no. 353015); three (3) BD Falcon™ 96-Well UV Transparent Microplates (BD cat. no. 353261), one (1) BD Falcon™ 96-well 2 mL Polypropylene Storage Plate (BD cat. no. 353966); three (3) 200 μ L and two (2) 1000 μ L disposable pipet tip racks; and one (1) “dummy” receiver plate was used to rest the PAMPA insert during access to the donor wells. Integrated to the robot are a UV Plate reader, temperature controlled incubator, and multiple hotel shelves.

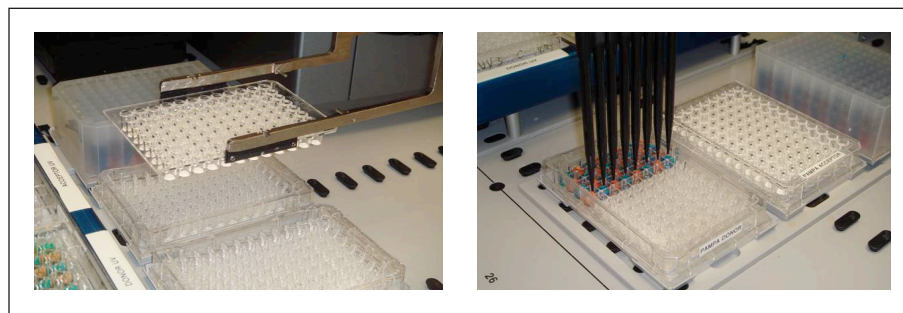


Figure 4. The Integrated Robot Gripper and Pipette Tips are Fully Compatible with the BD Gentest™ Pre-coated PAMPA Plate System.

The Tecan RoMa™ (robotic arm) was programmed with vectors to retrieve and manipulate individual components of the assembly (lids, receiver, and donor components). The RoMa also transferred the UV assay plates to the plate reader. Both 200 μ L and 1000 μ L conductive Tecan DiTi™ (disposable tips) were programmed to reproducibly aspirate and dispense with liquid level sensing and a 1 mm offset to minimize the risk of a membrane puncture or tear.

Presented as a Poster, Society for Biomolecular Sciences Meeting 2008

Automation of Pre-coated PAMPA Plates Improves Predictability, Reproducibility, and Efficiency

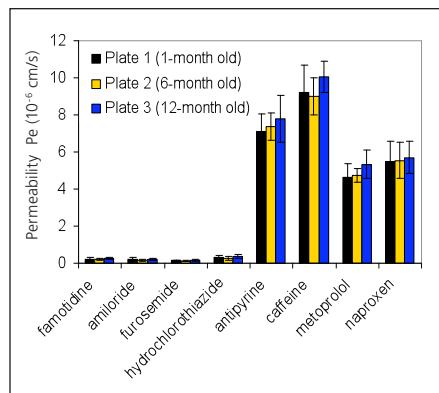


Figure 5. Reproducibility and Stability of theBD Gentest™ Pre-Coated PAMPA Plate System.

Comparison of PAMPA permeability values of 8 compounds obtained using 3 pre-coated plates prepared at different times: Plate 1 was prepared one month before the day of assay, Plate 2 was prepared 6 months before the day of assay, Plate 3 was prepared one year before the day of assay.

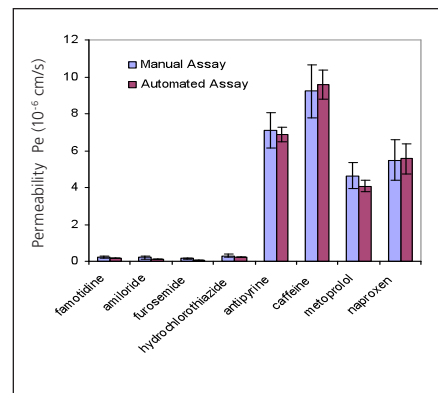


Figure 6. Automated Assay Improved CV Compared to Manual Assay.

Comparison of PAMPA permeability values of 8 compounds obtained using manual assay and automated assay methods. The automated assay method produced better CVs.

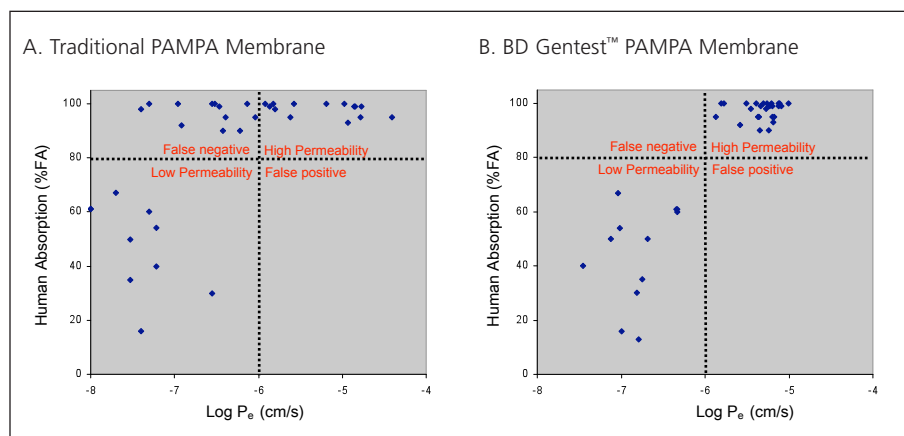


Figure 7. BD Gentest™ PAMPA Membrane Improves Correlation with Human Absorption Data.

Comparison of the performance of traditional PAMPA membrane and the BD Gentest™ PAMPA membrane by analyzing the correlation of the permeability data with the human absorption data for a set of 35 compounds. The permeability data of the traditional PAMPA membrane and the human absorption data were cited from Reference 3. The permeability data of the BD Gentest™ Pre-Coated PAMPA Plate System membrane were obtained using UV VIS measurements; both donor and acceptor buffers were PBS, pH 7.4; and the PAMPA plate system was incubated at room temperature for 5 hours without agitation.

Results

The BD Gentest™ Pre-coated PAMPA Plate System simplifies the PAMPA process since effort is not required to form and prepare the membrane prior to adding the compounds. **Figure 1** compares the traditional PAMPA process flowchart and the new PAMPA process flowchart. The traditional process requires the user to perform coating of the filter plate before setting up the assay. In the new process, the filter plate is ready to use, therefore the assay can be started by the addition of compounds to the Donor wells, addition of buffer to the Acceptor wells, and coupling the Donor and Acceptor plates together for incubation. Overall time required to run the assay is greatly reduced when compared to traditional methods.

The new artificial membrane is designed not only to improve the stability, but also to improve the PAMPA model. Traditional PAMPA membrane has incorrect predictions for some high permeability compounds, presumably due to the excessive amount of solvents present in the membrane structure. **Figure 2** compares the structure of the artificial membrane of the traditional PAMPA and the BD Gentest™ Pre-coated PAMPA Plate System. The traditional PAMPA membrane is prepared by adding a solution of lipids. As a result, the porous filter is soaked with the solvent of the lipids, creating a long permeation pathway for the compounds. In comparison, the BD Gentest™ Pre-coated PAMPA Plate System PAMPA membrane is prepared by constructing thin layers of lipids inside the porous filter, using volatile solvents which evaporate after coating. Therefore, the permeation pathway is much shorter and is a better mimic of the biological membrane.

The BD Gentest™ Pre-coated PAMPA Plate System is fully automation compatible. This is demonstrated by carrying out the entire assay process using a Tecan Genesis 150 liquid handler which is integrated to a plate reader using a robot arm. The automation deck layout is shown in **Figure 3** and examples of robotic handling are shown in **Figure 4**.

To validate the stability of the pre-coated plates, experiments have been carried out using plates coated at different times. **Figure 5** compares the results obtained from three plates coated at different times and used for assay on the same day. The results obtained from the one-year-old plate and the six-month-old plate are almost identical to the results obtained from a freshly coated plate. These results indicate that the BD Gentest™ Pre-coated PAMPA Plate System is stable for at least one year when stored at -20°C and is highly reproducible from plate to plate.

To validate the automation of the pre-coated plates, the results obtained using automated assays were compared to the results obtained using manual assays. The results shown in **Figure 6** confirmed that both methods yielded the same results, while the automated methods generated data with tighter CVs, presumably due to more accurate pipetting compared to manual pipetting.

Figure 7 compares the performance of a traditional PAMPA and the BD Gentest™ Pre-Coated PAMPA Plate System by analyzing the correlation of the permeability data with the human absorption data of 35 compounds. Using the traditional PAMPA, there is a group of compounds with high human absorption properties that are under-predicted (false negative). This group of compounds are correctly predicted using the BD Gentest™ Pre-Coated PAMPA Plate System.

Conclusions

The BD Gentest™ Pre-Coated PAMPA Plate System is fully automation compatible and improves the PAMPA model through the following characteristics:

- Pre-coated plates improve process efficiency because the membrane preparation step is skipped
- Pre-coated plates are stable and highly reproducible from plate to plate
- The new artificial membrane better mimics the biological membrane and improves PAMPA predictability, as demonstrated by the improved correlation with human absorption data

References

1. Kansy, M., Senner, F., Gubernator, K. *J. Med. Chem.* **41**:1007 (1998).
2. Avdeef, A., Strafford, M., Block, E., Balogh, M., Chambliss, W., Khan, I. *Eur. J. Pharm. Sci.* **14**:271 (2001).
3. Ruell, J.A., Avdeef, A., Du, C., Tsinman, K. A Simple PAMPA Filter for Passively Absorbed Compounds, Poster, ACS National Meeting, Boston, August 2002.
4. Chen, X., Murawski, A, Patel, K., Crespi, C. L., Balimane, P. *Pharmaceutical Research*, in press (2008)

**BD Biosciences**

Two Oak Park
Bedford, MA 01730 USA
tel: 877.232.8995
fax: 800.325.9637
bdbiosciences.com