A Novel Design of Artificial Membrane for Improving the PAMPA Model

Application Note

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Introduction

The Parallel Artificial Membrane Permeability Assay (PAMPA) is a well-accepted screening assay for ADME properties (membrane permeability). Since the first successful demonstration of PAMPA by Kansy, et al.,[1] the artificial membrane has usually been prepared by impregnating a porous filter with a solution of lipids and other biological membrane constituents. The artificial membranes in PAMPA must be both robust enough to generate reproducible results in a screening environment and provide a good model of the *in vivo* biological membrane. While the traditional method—forming artificial membranes using lipid solutions—seems to provide good predictability for many compounds, it is challenged by limited reproducibility and the incorrect prediction of a group of drugs that are classified by the biopharmaceutical classification system (BCS) as high permeability compounds. Our investigations suggested an excess amount of solvents and lack of structure of the artificial membrane may contribute to the underprediction of some high BCS permeability compounds. We have developed a novel lipid/oil/lipid tri-layer artificial membrane that does not contain excessive solvents. The pre-coated filter plates with the lipid/oil/lipid tri-layer artificial membrane (Corning® BioCoat™ Pre-coated PAMPA Plate System) has been evaluated in comparison with traditional PAMPA methods for its predictability, stability, reproducibility, ability to reduce mass retention, and compatibility with buffers containing organic solvents.

Materials and Methods

The Corning BioCoat Pre-coated PAMPA Plate System (Cat. No. 353015) was used to perform permeability assays for 38 commercially available drug compounds. The permeability assay was carried out in a similar protocol.[1-3] 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). As shown in Figure 1, 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 was calculated using the following formula:

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

A = filter area (0.3 cm^2), VD = 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 2 compares the structure of the artificial membrane of the traditional PAMPA and the lipid/oil/lipid tri-layer membrane of the Corning BioCoat Pre-coated PAMPA Plate System. The photo of coated and uncoated PVDF filters provides evidence the lipid/oil/lipid tri-layer membrane does not contain excessive solvents, while the traditional PAMPA membrane contains excessive solvents that make the PDVF filter semi-transparent.

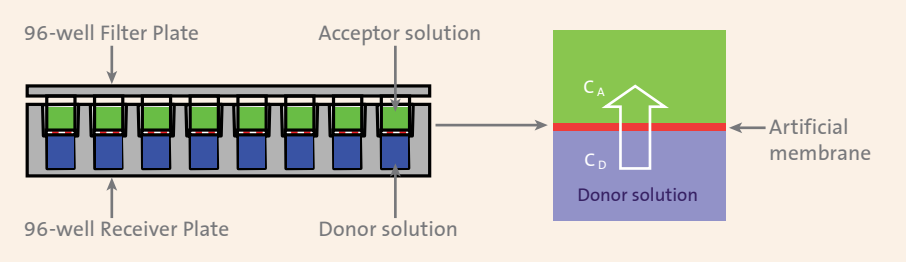

Figure 1. Experimental setup of PAMPA. Compound solutions are added to the receiver plate (donor) and buffer is added to the pre-coated filter plate (acceptor). The plates are coupled together and incubated at room temperature for 5 hours. During the incubation, compounds in the donor solution permeate through the artificial membrane into the acceptor solution. By measuring the compound concentrations—C_A and C_D—in both solutions, permeability of the compounds can be calculated.

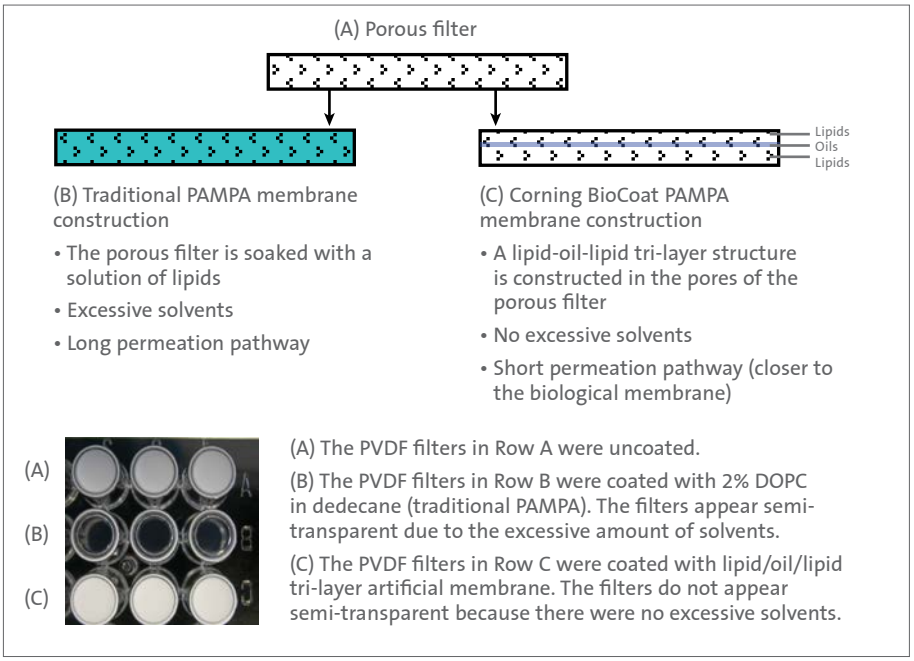

Figure 2. Comparison of the traditional PAMPA membrane and the Corning® BioCoat™ PAMPA membrane. Compound solutions are added to the receiver plate (donor) and buffer is added to the pre-coated filter plate (acceptor). The plates are coupled together and incubated at room temperature for 5 hours. During the incubation, compounds in the donor solution permeate through the artificial membrane into the acceptor solution. By measuring the compound concentrations—C_A and C_D—in both solutions, permeability of the compounds can be calculated.

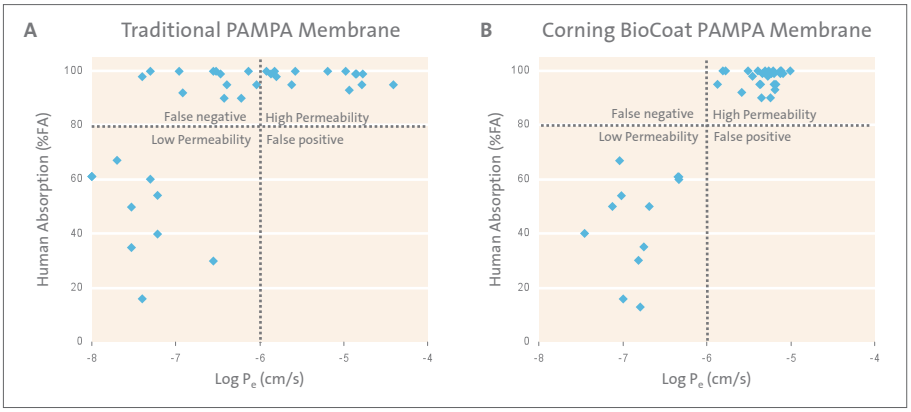

Figure 3. Corning BioCoat PAMPA membrane improves correlation with human absorption data. Comparison of the performance of traditional PAMPA membrane and the Corning BioCoat PAMPA membrane by analyzing the correlation of the permeability data with the human absorption data for a set of 38 compounds. The permeability data of the traditional PAMPA membrane and the human absorption data were cited.[3] The permeability data of the Corning BioCoat PAMPA 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.

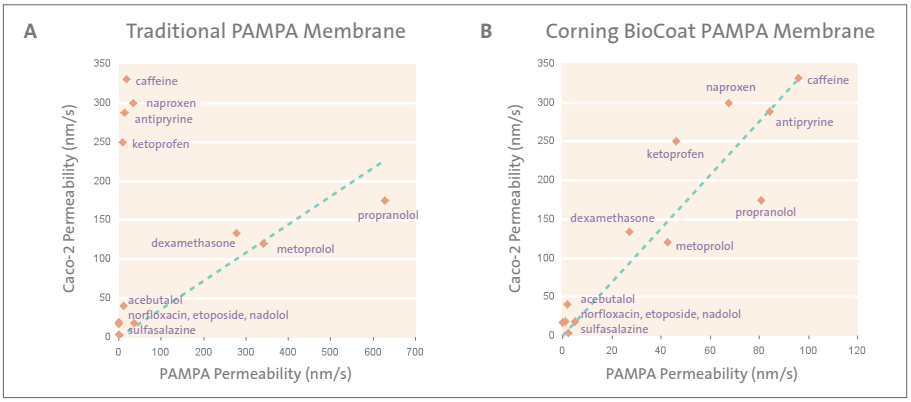

Figure 4. Corning BioCoat PAMPA membrane improves correlation with Caco-2 data. Comparison of the performance of traditional PAMPA membrane and the Corning BioCoat PAMPA membrane by analyzing the correlation of the permeability data with the Caco-2 permeability data for a set of 11 compounds. The permeability data of the traditional PAMPA membrane and the Caco-2 permeability data were cited.[4,5] The permeability data of the Corning BioCoat PAMPA 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 and Discussion

Predictability

Predictability of a PAMPA method is evaluated by the correlation with human absorption and Caco-2 data. Figure 3 compares the performance of a traditional PAMPA and the Corning® BioCoat™ Pre-coated PAMPA Plate System by analyzing the correlation of the permeability data with the human absorption data of 38 compounds. Using the traditional PAMPA, there is a group of compounds with high human absorption property that are underpredicted (false negative). This group of compounds are correctly predicted using the Corning BioCoat Pre-coated PAMPA Plate System. Figure 4 compares the performance of a traditional PAMPA and the Corning BioCoat Pre-Coated PAMPA Plate System by analyzing the correlation of the PAMPA permeability data with Caco-2 permeability data. Using the traditional PAMPA, there is a group of compounds with high Caco-2 values that are underpredicted, including antipyrine, caffeine, naproxen, and ketoprofen. This group of compounds were correctly predicted using the Corning BioCoat Pre-coated PAMPA Plate System. These results indicate that the new lipid/oil/lipid tri-layer artificial membrane improves the PAMPA predictability.

Stability and Reproducibility

Figure 5 compares the results obtained from three plates coated at different times and used for assays 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 Corning BioCoat 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.

Mass Retention

Some compounds can bind to the surface of the plates and/or be trapped inside the artificial membrane, resulting in high mass retention. Figure 6 compares the mass retention of three of these compounds using the traditional PAMPA and using the Corning BioCoat Pre-coated PAMPA Plate System. These results indicate the Corning BioCoat Pre-coated PAMPA Plate System reduces the mass retention of these compounds.

Compatibility with Organic Solvents

Low solubility compounds have been a challenge for permeability measurements. Using a buffer containing organic solvents helps to increase the solubility of these compounds. Figure 7 compares the permeability measurements of eight compounds in three buffer conditions: (1) PBS, (2) PBS +10% methanol, and (3) PBS +20% methanol. These results indicate that the artificial membrane has maintained its integrity and the correct ranking of the compounds can be obtained with buffers containing up to 20% methanol.

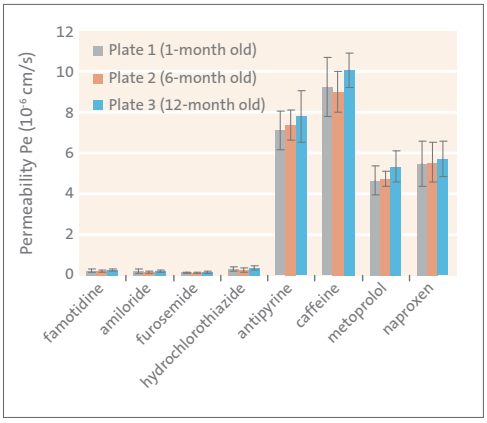

Figure 5. Reproducibility and stability of the Corning BioCoat pre-coated PAMPA plate system. Comparison of PAMPA permeability values of 8 compounds obtained using 3 pre-coated plates prepared at different times:
Plate #1 was prepared one month before the day of assay;
Plate #2 was prepared 6 months before the day of assay;
Plate #3 was prepared one year before the day of assay.

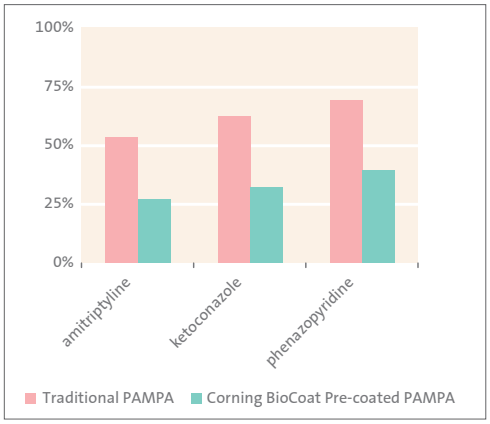

Figure 6. Corning BioCoat pre-coated PAMPA plate system reduces mass retention of "sticky" compounds. Comparison of the mass retention of amitriptyline, ketoconazole, and phenazopyridine using traditional PAMPA and the Corning BioCoat pre-coated PAMPA plate system. Data of the traditional PAMPA are from Reference 2. Mass retention is calculated by:
$R = 1 - [C_D(t)*V_D+C_A(t)*V_A]/(C_0*V_D)$,
where C_0 is the initial compound concentration in donor well.

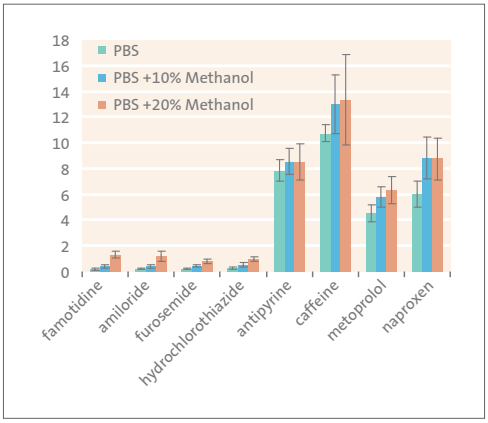

Figure 7. Compatibility of the Corning BioCoat pre-coated PAMPA plate system with buffers containing organic solvents. Comparison of PAMPA permeability values of 8 compounds obtained using 3 buffer conditions:
Condition #1 used PBS as the buffer for both donor and acceptor solutions;
Condition #2 used PBS +10% methanol as the buffer for both donor and acceptor solutions;
Condition #3 used PBS +20% methanol as the buffer for both donor and acceptor solutions.

Conclusions

- The Corning® BioCoat™ Pre-coated PAMPA Plate System, which contains a novel lipid/oil/lipid tri-layer artificial membrane, improves the PAMPA model through the following characteristics:
  - Improved correlation with human absorption data
  - Improved correlation with Caco-2 data
  - Stability for more than one year from date of manufacture when stored at -20°C
  - Highly reproducible results obtained from plates coated at different times
  - Reduced mass retention of "sticky" compounds
  - Compatibility with buffers containing organic solvents (to improve low solubility compounds)

Acknowledgement

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References

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