A Novel Design of Artificial Membrane for Improving the PAMPA Model

Application Note 479

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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., (Reference 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® Gentest™ 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 Gentest 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 as described in References 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 UV-plate reader. Permeability of the compounds was calculated using the following formula:

\[ P_e = \frac{-\ln[C_A(t) / C_{eq}]}{A \times (1/V_D + 1/V_A) \times t} \]

where:
- \( A \) = filter area (0.3 cm²),
- \( V_D \) = donor well volume (0.3 mL),
- \( V_A \) = acceptor well volume (0.2 mL),
- \( C_A(t) \) = compound concentration in acceptor well at time \( t \),
- \( C_D(t) \) = compound concentration in donor well at time \( t \), and
- \( C_{eq} = \frac{[C_D(t) \times V_D + C_A(t) \times V_A]}{(V_D + V_A)} \)

Figure 2 compares the structure of the artificial membrane of the traditional PAMPA and the lipid/oil/lipid tri-layer membrane of the Corning Gentest 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 five hours. During the incubation, compounds in the donor solution permeate through the artificial membrane into the acceptor solution. By measuring the compound concentrations—CA and CD—in both solutions, permeability of the compounds can be calculated.

Figure 2. Comparison of the Traditional PAMPA Membrane and the Corning Gentest 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 five hours. During the incubation, compounds in the donor solution permeate through the artificial membrane into the acceptor solution. By measuring the compound concentrations—CA and CD—in both solutions, permeability of the compounds can be calculated.

Figure 3. Corning Gentest PAMPA Membrane Improves Correlation with Human Absorption Data
Comparison of the performance of traditional PAMPA membrane and the Corning Gentest 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 from Reference 3. The permeability data of the Corning Gentest 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 Gentest PAMPA Membrane Improves Correlation with Caco-2 Data
Comparison of the performance of traditional PAMPA membrane and the Corning Gentest 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 from References 4-5. The permeability data of the Corning Gentest 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.
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 Gentest 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 Gentest Pre-Coated PAMPA Plate System.

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 Gentest Pre-Coated PAMPA Plate System. These results indicate that the Corning Gentest 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.
Conclusions

- The Corning® Gentest™ 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 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)

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References