Interpreting In Vitro Permeation Testing Layer-Deposition Data Using a Mechanistic Skin Model in MoBi

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ABSTRACT

Background and Purpose: Predicting the bioavailability of a chemical following topical application or exposure is essential for assessing the risk and efficacy of dermatological products. In vivo studies using preclinical species are often constrained by cost, complexity, and limited translational relevance. As a result, new approach methodologies (NAMs), including in vitro and in silico tools, are increasingly relied upon in toxicological evaluations. In vitro permeation testing (IVPT) with excised human skin can be combined with mechanistic skin permeation models to extrapolate these data toward in vivo predictions. However, refining the implementation and understanding the limitations of such models is necessary before they can be widely adopted for regulatory purposes. In this study, IVPT data were compared with simulated results generated using a skin permeation model implemented in MoBi, part of the Open Systems Pharmacology suite. Additionally, layer-deposition data from both experimental and simulated datasets were analyzed to extrapolate the in vivo bioavailability of the test compounds.

Methods: IVPT was performed on four test articles, caffeine (CAS No. 58-08-2, MW 194.19 g/mol, log K_{ow} -0.07, salicylic acid (CAS No. 69-72-7, MW 138.12 g/mol, log K_{ow} 2.26), testosterone (CAS No. 58-22-0, MW 288.43 g/mol, log K_{ow} 3.32), and mannitol (CAS No. 69-65-8, MW 182.17 g/mol, log K_{ow} -3.10). Excised human skin (LifeNet Health; cryopreserved, cadaver, leg or back, 500 µm dermatomed) was mounted on Franz diffusion cells (PermeGear; 0.64 cm^2 active area) connected to a heated water circulator system. Skin temperature and barrier integrity were measured by infrared thermometer and transepidermal water loss (Delfin VapoMeter), respectively. A finite dose (10 µL/cm^2) of test article (1% w/v in buffer) was applied to the apical skin surface, and receptor fluid (DPBS) collections were performed at 0, 0.5, 1, 2, 3, 4, 6, and 24 hours post-dosing. Apical wash, *stratum corneum* tape strips (three per sample), and tissue were collected following the final (24 hour) receptor fluid sampling. All samples were extracted and analyzed by liquid chromatography tandem mass spectroscopy (LC-MS/MS). In silico predictions of chemical dermal absorption were collected in parallel. Test article physicochemical properties and test system parameters were input into the MoBi (version 12.0.434) implementation of the (Dancik, Miller et al. 2013) skin permeation model (https://github.com/Open-Systems-Pharmacology/Skin-permeation-model) and simulated cumulative permeation, flux, and layer deposition data were generated.

Results: The Mean ± SEM 24-hour cumulative absorption observed in the in vitro permeation test ranked from lowest to highest as follows: mannitol (no detectable absorption), testosterone (2.53 ± 0.20 µg/cm^2), salicylic acid (7.70 ± 3.93 µg/cm^2), caffeine (14.84 ± 5.30 µg/cm^2). The simulated mean 24-hour cumulative absorption, alternatively, ranked from lowest to highest as follows: mannitol (2.07x10^{-29} µg/cm^2), caffeine (0.87 µg/cm^2), testosterone (1.75 µg/cm^2), salicylic acid (99.92 µg/cm^2). For testosterone, the model layer deposition data predicted 96.69% of the applied dose would remain on the apical skin surface versus the experimentally observed 91.62 ± 3.40%. Simulated versus experimental comparisons of apical retention for the remaining test articles were as follows: caffeine = 41.94% vs 70.86% ± 5.32%, salicylic acid = 0.06% vs 3.02% ± 0.18%, mannitol = 57.98% vs 20.85% ± 10.24%.

Conclusions: The mechanistic skin model was limited in its ability to accurately predict the cumulative absorption of the test compounds. The layer deposition data generated, however, was more accurate at correctly ranking the results observed experimentally. This study underscores the value of mechanistic skin permeation models in refining in vivo dermal absorption predictions. Integrating IVPT data with computational models can strengthen predictive accuracy and increase confidence in risk assessments. Conversely, computational models can contextualize experimental findings, offering deeper insights into observed results. In both scenarios, employing multiple NAMs within a weight-of-evidence framework enhances the robustness and regulatory relevance of the assessment.

Figure 1. Diagrammatic Representations of the Principles of the In Vitro Permeation Test and Mechanistic Skin Model

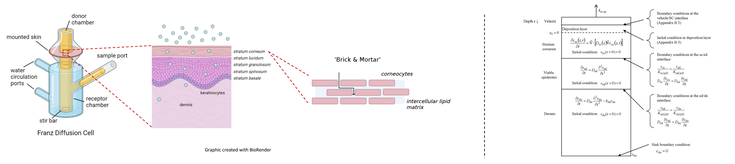

Fig. 1 Diagrammatic representations of the principles of the in vitro permeation test and mechanistic skin model. (Left) The dermal absorption of a test article may be evaluated in vitro, through excised skin mounted on a diffusion cell (e.g. static Franz diffusion cell). The lipid-rich *stratum corneum* provides a selective barrier to permeation and, together with the viable epidermis, regulates systemic exposure of topically applied compounds. (Right) The mechanistic skin model (from (Dancik, Miller et al. 2013)) simulates one-dimensional transient passive transport into a skin slab, separated into three layers: the *stratum corneum*, the viable epidermis, and the dermis.

INTRODUCTION

In vitro permeation testing (IVPT) is a method used to evaluate the permeation characteristics of topical drug products, cosmetic ingredients, and environmental exposures through the skin (Fig. 1; (Salminen, et al. 2023)). This assay can be used alongside in vivo methods to support regulatory submissions for topical drugs, such as in FDA's Abbreviated New Drug Applications (ANDAs) for bioequivalence demonstrations. For cosmetic ingredients and environmental chemicals, IVPT provides crucial data for risk assessments to ensure consumer and public safety.

In silico tools have become essential in modern toxicology, offering fast, cost-effective, and mechanistically-informed ways to predict chemical hazards without relying solely on traditional laboratory testing. **Mathematical models of skin permeability** have played a large role in the field of transdermal drug delivery and cosmetics and occupational risk assessments. Potts and Guy developed the most widely-adopted quantitative structure-property relationship (QSPR) model for predicting skin permeability in 1992 (Potts & Guy, 1992):

$$\log k_p = -6.3 + 0.71 \log K_{ow} - 0.0061 MW$$

INTRODUCTION Cont.

where k_p is the skin permeability coefficient (cm/s), log K_{ow} is the octanol-water partition coefficient, and MW is the molecular weight. Since this seminal equation was established, decades of research have led to the development of numerous steady-state and transient skin permeability models, including the model developed by (Dancik, Miller et al. 2013) (Fig. 1). Together, mathematical models of skin permeability and IVPT exist as powerful new approach methodologies (NAMs) with the potential to advance our understanding of transdermal delivery and risk following dermal exposure.

IVPT MATERIALS & METHODS

Materials

Test Articles: Caffeine (CAS No. 58-08-2), Salicylic acid (CAS No. 69-72-7), Testosterone (CAS No. 58-22-0), and Mannitol (CAS No. 69-65-8) were purchased from Sigma and formulated in 1X Dulbecco's Phosphate-Buffered Saline (1X DPBS) at 1% w/v (10 mg/mL), with the exception of testosterone, which was formulated in pure ethanol.

Extraction Fluid: 50% v/v methanol in Ultrapure water was prepared using Methanol anhydrous, 99.8% purchased from Sigma and Ultrapure water purchased from ThermoFisher.

Receptor Fluid: 1X DPBS with calcium and magnesium was purchased from Corning.

Wash Fluid: 200 Proof Ethanol was purchased from Decon Labs.

Tape Stripping: D-Squame Stripping Discs and a D-Squame Pressure Instrument were purchased from Clinical & Derm.

IVPT Methodology

The in vitro permeation test was performed in accordance with OECD Test Guideline 428. Franz-cell (PermeGear) mounted skin temperature (32 ± 1°C) was verified after equilibration of the system, prior to dosing, with an infrared thermometer. Transepidermal water loss (TEWL) was measured across Franz cell-mounted skin with a VapoMeter (Delfin Technologies), immediately prior to dosing, to confirm skin integrity.

All test compounds were applied in a finite dose (10 µL/cm^2) and remained on the skin (Fig. 2; 500 µm, cadaver, leg or back; LifeNet Health) for the duration of the experiment (24-hour exposure). Receptor fluid collections were made at 0-, 0.5-, 1-, 2-, 3-, 4-, 6-, and 24-hours post-application of the test article. Following experimentation, the apical skin surface was washed to remove un-absorbed test article. Wash fluid was collected with a cotton pad. Tape strips were collected (three per tissue), and tape strips, cotton pads (wash) and viable epidermis + dermis were extracted in 50% v/v methanol in Ultrapure water (extraction fluid).

Bioanalytical Testing

Bioanalytical testing to quantify analyte in the receptor fluid collections and tape strips, wash and viable epidermis + dermis extracts was performed using a Shimazdu Nexera XR LC-30AD UPLC system in-line with a SCIEX 6500+ mass spectrometer via an electrospray ionization (ESI) interface (i.e. LC-MS/MS) with a 1 ng/mL (testosterone), 5 ng/mL (caffeine), 10 ng/mL (salicylic acid) or 50 ng/mL (mannitol) limit of quantitation.

Data Analysis

The cumulative amount of analyte in the receptor fluid was calculated from the LC-MS/MS results for each collection (n) as follows:

When $n = 1, Cumulative\ Amount = (RF_n)V_R$

When $n > 1, Cumulative\ Amount = (RF_n)V_R + \left(\sum_{i=1}^{n-1} RF_i \times V_S\right)$

Where RF_n is the concentration of analyte in receptor fluid sample (n), V_R is the total volume of the receptor compartment (5 mL), and V_S is the volume of the sample (500 µL). Sample n = 1, 2, 3, 4, 5, 6, 7, 8 corresponds to receptor fluid collections at 0, 0.5, 1, 2, 3, 4, 6, and 24 hours post-dosing, respectively. Cumulative amount was normalized to the surface area of the Franz diffusion cell orifice (0.64 cm^2) and reported as µg/cm^2.

Flux, reported as µg/cm^2/h, was calculated by dividing the surface area-normalized amount of analyte permeated between collection times by the time between collections.

Mass distribution of the analyte was calculated for each compartment; tape strip 1, 2, and 3; wash; viable epidermis + dermis; and receptor; by dividing the total amount of analyte in each compartment at 24 hours post-dosing by the amount of analyte dosed.

Figure 2. Histology micrograph of excised human skin.

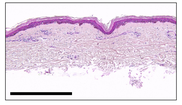

Fig. 2 Histology micrograph of excised human skin. Human donor skin was excised and dermatomed to a uniform target thickness of 500 µm. Skin sections were collected and processed for slide mounting and H&E staining. Micrographs were collected on a BioTek Cytation 5. Scale bar = 500 µm. Representative image shown. Donor characteristics: female, 74 years of age, Asian, frozen, cadaver, warm ischemic time = 16.47 hours, leg or back.

RESULTS

Figure 3. Example Input Parameters and Simulation Results in MoBi.

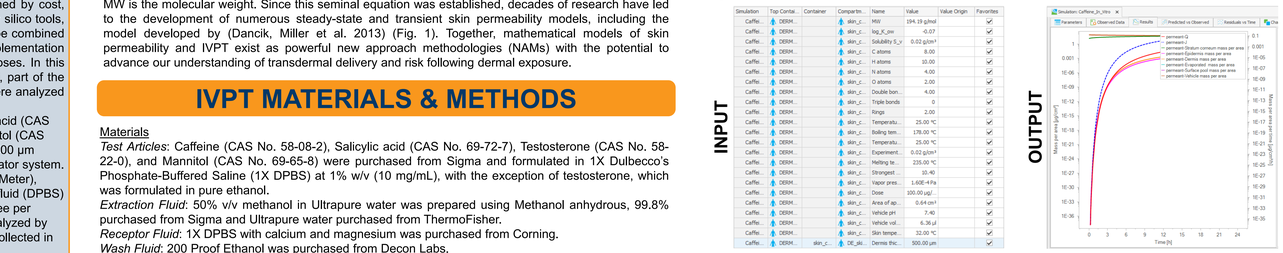

Fig. 3 Example input parameters and simulation results in MoBi. The mechanistic skin model from (Dancik, Miller et al. 2013) was loaded into MoBi Version 12 – Build 434. Parameters, including but not limited to, excised skin characteristics, experimental conditions, and permeant physicochemical properties were input into the model and simulated results (e.g. permeant cumulative permeation, flux, and mass distribution) were generated for comparison to experimental data. Example inputs and outputs for caffeine are shown.

Figure 4. Comparison of Experimental and Simulated Cumulative Amount, Flux, and Mass Distribution.

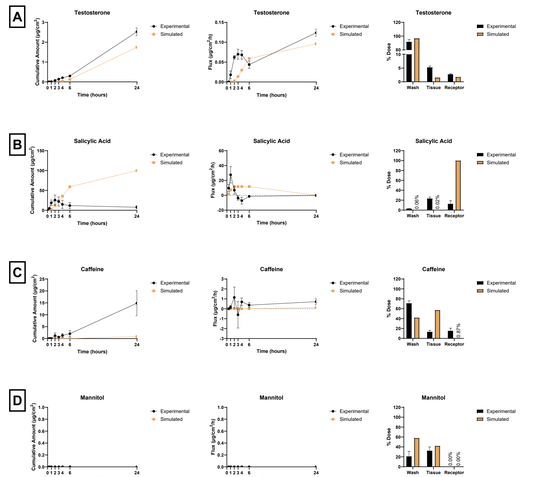

Fig. 4 Comparison of experimental and simulated cumulative amount, flux, and mass distribution. Dermal absorption of a finite dose (10 µL/cm^2) of (A) testosterone (1% w/v in pure ethanol), (B) salicylic acid (1% in 1X DPBS), (C) caffeine (1% in 1X DPBS), or (D) mannitol (1% in 1X DPBS) was assessed through excised human skin. Receptor fluid collections were performed at 0, 0.5, 1, 2, 3, 4, 6, and 24 hours post-application of the test article. At 24-hours post-dosing, the apical skin surface was washed, and the skin (three tape strips + viable epidermis and dermis) was collected. Permeant amount in each sample was quantified by LC-MS/MS. Cumulative amount (left), flux (middle), and mass distribution (right) were calculated from LC-MS/MS results. N = 3 independent Franz cells, one skin donor. Mean ± standard error of mean are presented.

Figure 5. Experimental Versus Simulated IVPT Layer-Deposition Data

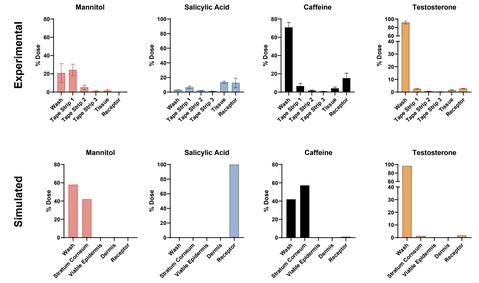

Fig. 5 Experimental versus simulated IVPT layer-deposition data. Layer-deposition data for each test article (mannitol, salicylic acid, caffeine, and testosterone) were collected experimentally (top) or derived using the mechanistic skin model in MoBi (bottom). Comparison of results, together with a thorough understanding of the mechanistic skin model's limitations, can aide in interpreting the experimental data towards extrapolating these findings to an in vivo prediction. Experimental Tape Strips 1 + 2 + 3 = Simulated Statum Corneum; Experimental Tissues = Simulated Viable Epidermis + Dermis.

CONCLUSIONS

- The MoBi implementation of the (Dancik, Miller et al. 2013) mechanistic skin model varied in its ability to accurately predict the dermal absorption of the four test articles, as observed in the in vitro permeation test.
- Simulated IVPT layer-deposition results aid in the contextualization of the corresponding experimental data, informing on potential routes of skin entry across physicochemical properties.
- The opensource MoBi platform is a user-friendly in silico tool for enhanced skin permeability assessments.
- Combining in vitro and in silico NAMs for toxicological assessments can further strengthen the regulatory relevance of these novel tools.

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Skin Permeation Model Code