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Review article

Evaluating drug permeability: a comparative study of *in vitro* permeability models and their regulatory applications

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ABSTRACT

To make oral & transdermal drug products, we want to know a lot about how active pharmaceutical ingredients (APIs) can get through the skin, which is a biological barrier that is very complicated. The very reliable non-clinical technique for quantitatively evaluating drug delivery from semi-solid formulations is the in vitro permeation test (IVPT). IVPT determines how quickly & thoroughly a medication penetrates into skin, gets accessible at the site of action. This technique produces important endpoints like Flux and Cumulative Amount Permeated using serial sampling and physiological circumstances. In order to support the evidence of bioequivalence for complex topical generics, regulatory agencies like the EMA and FDAare increasingly recommending IVPT studies. Drug penetration occurs via the Trans-epidermal (transcellular and intercellular) and Trans-appendageal routes, with absorption influenced by factors like the Stratum Corneum barrier function, drug properties (e.g., lipophilicity, size), and formulation excipients. Beyond excised skin, other in-vitro permeability models include cell-based systems such as Human Intestinal Cell (Caco-2) and Canine Kidney Cell (MDCK) and non-cell-based high-throughput techniques like parallel artificial membrane permeability assay (PAMPA), and the Franz diffusion cell. Future advancements focus on addressing challenges like membrane variability and sink conditions through technologies such as Raman microspectroscopy, 3D bioprinted skin models, machine learning integration (ML), and Artificial Intelligence.

Keywords: In-Vitro Permeation Test (IVPT), Bioequivalence, PAMPA, Skin.

INTRODUCTION

The development of topical and transdermal drug products, which include semi-solid formulations like creams, gels, ointments, and patches, requires a robust understanding of how the active pharmaceutical ingredient (API) is delivered to or through the skin. The skin, a highly complex and intricate organ, serves as the primary barrier to external agents, making percutaneous absorption a uniquely challenging pharmacokinetic process. Over the past few decades, In Vitro Permeation Test (IVPT), also known as the *in vitro* skin penetration/permeation test, has emerged as the definitive and most widely accepted methodology for the quantitative assessment of drug delivery from these topical formulations [1].

The IVPT is a critical, non-clinical tool used across the drug development lifecycle. Its primary purpose is to measure the rate and extent to which an API permeates through a biological membrane, typically excised skin, and becomes available locally at the site of action or enters the receptor medium simulating systemic circulation.

In Vitro Permeation Test (IVPT) is a test designed to mimic biological conditions. It is primarily used for product development. IVPT studies replicate the conditions of human skin to measure the rate at which drug ingredients penetrate through the skin layers. This method provides valuable data on the absorption, distribution, and potential efficacy of topical products without the need for preliminary

human trials. IVPT technique is widely used in the pharmaceutical, cosmetic, and personal care industries to optimize formulations. The IVPT membrane is typically excised human skin. The IVPT receptor solution/medium is typically a physiological buffer. The IVPT methodology maintains the skin at physiological conditions [2].

The experiment involves serial sampling of the receptor medium over time. The key endpoints derived from the drug concentration versus time data are:

Flux

The rate of drug permeation, typically expressed in units of mass per area per time.

Cumulative amount permeated

The total amount of drug that has passed through the skin and into the receptor medium over the study duration [3].

How IVPT Differs from IVRT

In Vitro Release Test (IVRT) is a test designed to determine product quality. It is primarily used for quality control of semisolids [4]. IVPTs and IVRTs serve very different purposes. The IVPT helps determine how the product will behave when applied. The IVRT is a quality control tool that monitors batch-to-batch uniformity. The IVPT membrane is typically excised from human skin.

In recent years, the regulatory significance of IVPT has dramatically increased, particularly for generic drug products. Regulatory bodies like the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) now recommend IVPT studies to support the demonstration of Bioequivalence (BE) for many complex topical products.

Table 1: Difference Between IVPT and IVRT

Feature	In Vitro Permeation Test (IVPT)	In Vitro Release Test (IVRT)
Primary Goal	Performance & Bioequivalence (BE). Mimics in vivo biological absorption to assess the rate and extent of drug delivery to or through the skin.	Quality Control (QC) & Formulation Consistency. Measures the rate of drug release from the formulation matrix.
Membrane Used	Biological Membrane (e.g., excised human or animal skin). The physiological barrier must be intact.	Synthetic/Inert Membrane (e.g., various polymer membranes). The membrane should be non-interactive.
Dosing	Finite Dose (thin-film) is typically applied and often not occluded, mimicking clinical use and allowing for formulation metamorphosis (drying).	Pseudo-Infinite Dose is used to maintain a consistent concentration at the membrane-formulation interface for steady-state kinetics. Often occluded.
Receptor Medium	Physiological Buffer (e.g., PBS with or without a small amount of solubility enhancer like BSA) to mimic biological sink conditions.	Organic/Aqueous Mixture (solvent/buffer mix) to ensure maximum solubility (high sink conditions) and reproducibility.
Data/Endpoints	Flux profile (μg/cm^2/h), Cumulative amount permeated (μg/cm^2), and Drug in skin layers (retention).	Release rate (e.g., concentration vs. time profile).
Regulatory Role	Pivotal for demonstrating Bioequivalence for complex generics (cutaneous pharmacokinetics).	Required for demonstrating product Quality (batch-to-batch consistency) and often used as the Q4 measure (Product Performance).

Principles of drug penetration

Skin is a readily accessible organ on human body for topical administration. It is the main route of topical drug delivery systems. Topical drug delivery refers to the treatment of a localized area of the skin. Transdermal drug delivery is a method designed to deliver drugs through the skin and into the systemic circulation. There are primarily two established pathways by which pharmacological agents can penetrate Trans epidermal Route and Trans appendageal Route.

Trans epidermal route

The trans epidermal route involves the movement of substances across the cellular matrix of the skin. This pathway can be divided into (i) transcellular and (ii) intercellular mechanisms. Transcellular permeation involves the direct absorption of drugs into and through individual skin cells. Transcellular permeation is efficient for hydrophobic compounds due to the lipid-rich interaction of the cell membranes. On the other hand, intercellular absorption occurs through the extracellular matrix by moving through the interstitial spaces between adjacent cells. The intercellular matrix is highly lipophilic hence this mechanism is also particularly favourable for hydrophobic drugs and is considered as the predominant mode of dermal drug absorption.

Trans appendageal route

The trans appendageal route involves drug penetration through skin appendages such as hair follicles and sebaceous glands. This route is favourable for the delivery of polar or ionizable drugs, as well as macromolecules that may be hindered by the dense structure of the stratum corneum. The presence of these appendages facilitates alternative pathways that favor the passage of larger and more complex drug molecules, thus increasing the range of compounds that can be effectively absorbed [5].

Skin is made up of several layers with different composition and structure. They are as follows from the deepest to the skin surface:

Hypodermis

Hypodermis is made of fatty tissues (adipocytes)

Dermis

Dermis is a hydrophilic layer irrigated by the blood circulation and composed of a gel in which a densely network of fibers (collagen and elastin) provides mechanical strength to skin. Any substance reaching it can pass into the systemic circulation

Epidermis

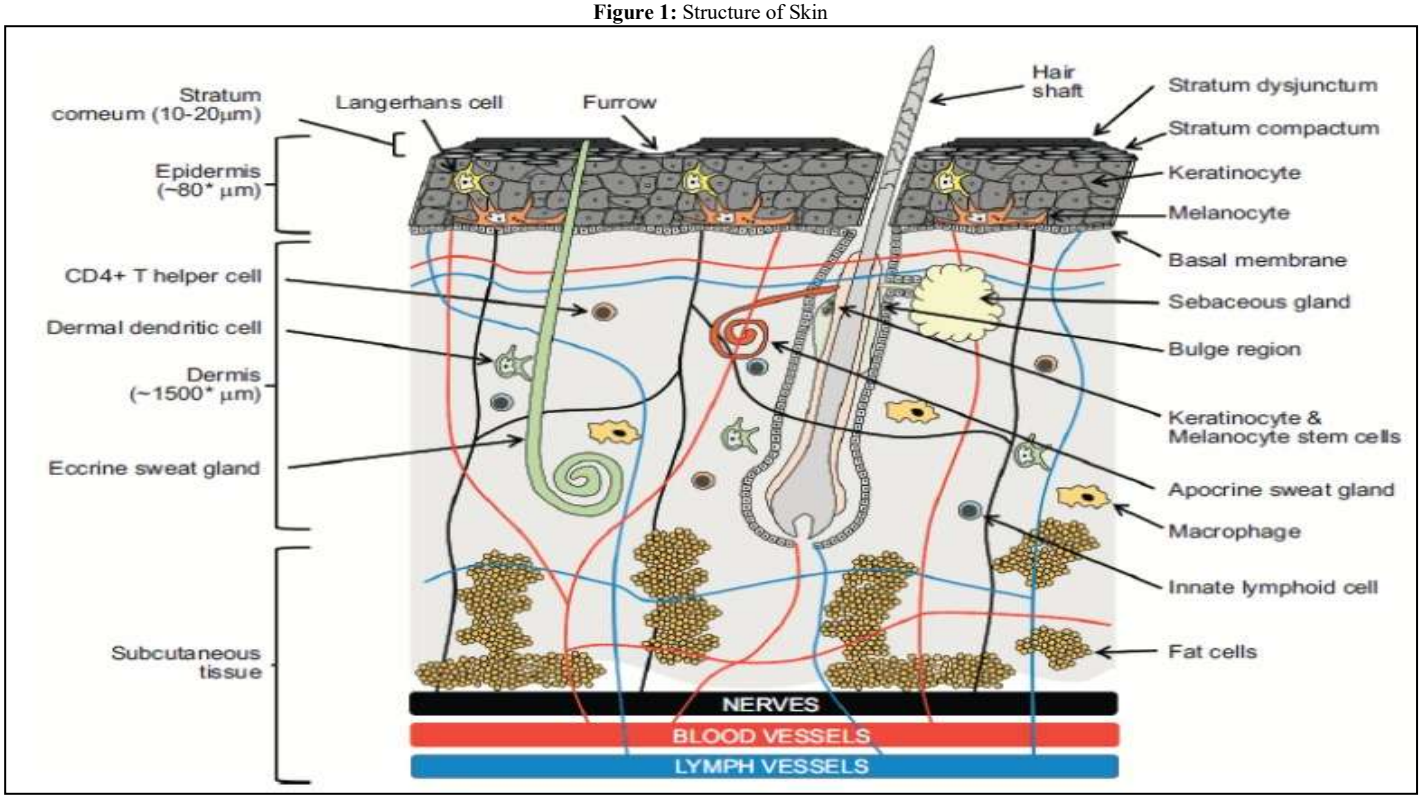
Epidermis is an epithelium divided into two distinct parts: the viable epidermis, a living hydrophilic layer (approx. 70% of water), and the *stratum corneum* (SC), a hydrophobic layer (approx. 13% of water) made from dead cells, resulting in a horny texture.

Stratum corneum

Stratum corneum is the layered structure of skin. It is

continuously renewed and provides efficient protection against the penetration of foreign substances. A hydrophilic substance cannot penetrate the skin easily because it cannot enter the hydrophobic SC layer. A hydrophobic substance easily enters the SC but it remains stored inside it since the next layer is hydrophilic. The outermost stratum corneum layer determines the barrier properties of the skin and it regulates the fluxes of chemicals and water between the environment and the organism [6].

Figure 1: Structure of Skin



Factors that affect drug penetration

There are three major factors that influence the skin absorption of drugs are skin barrier function, drug properties, and formulation factors. The skin acts as a barrier. It preventing the entry of harmful substances into the body. The outermost layer of the skin i.e. stratum corneum, plays a vital role in regulating the absorption of drugs. The condition and integrity of the skin barrier greatly impact drug absorption. Skin conditions such as dryness, cuts, burns, or dermatological disorders can compromise the skin barrier function and leads to increase in drug absorption. Other parameters like age, gender, and ethnicity can also influence the structure and permeability of the skin barrier. Drug properties significantly affect its ability to be absorbed through the skin. Some drugs are readily absorbed while others have limited absorption. The following properties play a crucial role. The smaller the molecule the higher the chance of permeation. Lipophilic (fat-soluble) drugs are more likely to cross the skin barrier and absorbed compared to hydrophilic (water-soluble) drugs. Lipid-soluble, non-ionized drugs have better absorption potential. Concentrated drugs have higher chance of skin absorption up to a certain level. Drug formulation plays an important role in the absorption of drug. The penetration of drug through the skin barriers can be enchased by optimizing vehicles used in formulation, using penetration enhancers like fatty acids, alcohols, and surfactants that increase the permeability of the skin barrier, allowing for better drug absorption. Different drugs exhibit better absorption at specific pH ranges. Excipients can also influence drug absorption by affecting the solubility, stability, or release of the drug [7].

***In-vitro* permeability models/methods**

Cell-based models

Caco-2

Caco-2 cells are human-derived intestinal epithelial cells originating from colorectal adenocarcinoma. These cells are widely employed in in-vitro permeation tests (IVPT) to predict oral drug absorption and intestinal permeability. The FDA recommends Caco-2 cell monolayers as an integral component of the Biopharmaceutics Classification System (BCS) to evaluate intestinal drug absorption. The apparent permeability coefficient values obtained in Caco-2 assays correlate well with the Fraction Absorbed in humans. Compounds are often categorized based on their apparent permeability coefficient to predict their classification in the Biopharmaceutics Classification System (BCS) [8].

Caco-2 cells express cytochrome P450 enzymes, efflux transporters (particularly P-glycoprotein), and uptake transporters. Cells form brush border-like structures similar to intestinal epithelium. Caco-2 cells are typically cultured on permeable supports (Transwell inserts) for 21 days to achieve full differentiation and monolayer maturation. The cells express transporters, metabolizing enzymes, and

form functional tight junctions. Drug transport is measured across the confluent monolayer mounted in diffusion chambers. Temperature is maintained at 37°C, and pH-appropriate buffers simulate physiological conditions.

Combining Caco-2 cells with mucus-producing HT29-MTX cells creates a more physiologically relevant barrier with a mucus layer. These models better replicate in vivo conditions, including the mucus layer's protective function. Caco-2 permeation studies support bioequivalence assessments for topical and poorly soluble drugs where traditional in vivo studies are challenging. The FDA increasingly accepts IVPT data using Caco-2 cells for certain drug classifications. Caco-2 cells are employed for multiple purposes.

Absorption prediction

The apparent permeability values predict human intestinal fraction absorbed (Fa) values. Studies show good correlation between Caco-2-derived permeability and observed human absorption, particularly for passive diffusion compounds.

Transporter identification

Caco-2 cells allow evaluation of carrier-mediated transport and efflux mechanisms. A bidirectional transport study distinguishes in the middle of passive diffusion and active transport.

Formulation evaluation

The model assesses how different drug delivery systems (nanoparticles, liposomes, polymeric carriers) enhance drug absorption.

Permeation enhancer screening

Caco-2 monolayers can evaluate chemical permeation enhancers and their mechanisms of tight junction modulation [9].

MDCK

The Madin-Darby canine kidney (MDCK) cell line is certainly one of the most widely utilised in vitro models for drug permeability screening in pharmaceutical development. Originally derived from canine kidney epithelium, MDCK cells form polarised monolayers with well-defined tight junctions, making them suitable surrogates for assessing intestinal and blood-brain barrier permeability [10].

Unlike human-derived cell lines, MDCK cells offer distinct advantages, including shorter culture periods and robust barrier formation. Two principal MDCK strains exist with markedly different barrier properties:

MDCK Strain I

Forms tight monolayers with high transepithelial electrical resistance (TEER), typically exceeding 1000 Ω·cm². This strain exhibits limited paracellular permeability, making it potentially suitable for studying blood-brain barrier-like conditions.

MDCK Strain II

Displays leakier tight junctions with lower TEER values. The reduced barrier integrity can result in overestimation of paracellular permeability, particularly for hydrophilic compounds [11].

MDCK cells are typically cultured on permeable Transwell supports for 3-7 days to achieve confluence and functional polarization. This relatively short culture period represents a significant advantage over Caco-2 cells, which require 21 days for full differentiation.

Applications in drug development

Formulation screening

MDCK cells evaluate how excipients, nanocarriers, and permeation enhancers affect drug absorption. Studies with chitosan nanoparticles demonstrated enhanced permeability through tight junction modulation.

Blood-brain barrier prediction: MDCK-pHaMDR cells predict CNS penetration for neurotherapeutics. The model correlates well with in vivo brain distribution data.

non-Cell-based models

Parallel artificial membrane permeability assay (PAMPA)

The Parallel Artificial Membrane Permeability Assay (PAMPA) is a non-cell-based, high-throughput screening (HTS) technique widely used in early drug discovery to predict the passive transcellular permeability of drug candidates. Introduced by Kansy and co-workers in 1998. The Parallel Artificial Membrane Permeability Assay (PAMPA) has emerged as a fundamental tool in pharmaceutical development and drug discovery. This cell-free, high-throughput technique offers a cost-effective alternative to traditional cell-based permeability assays while providing reliable predictions of passive drug absorption across biological membranes [12].

PAMPA is a non-cellular in vitro permeability model designed to evaluate drug absorption without the complexities and variability associated with cell culture systems. The assay employs an artificial lipid-based membrane that separates donor and acceptor compartments, allowing researchers to measure the rate at which compounds permeate across this barrier over defined time periods, typically 4-16 hours. The fundamental principle underlying PAMPA relies on passive diffusion through lipid membranes. As an early-phase drug candidate screening tool, PAMPA has been adopted as the primary screening method to assess passive diffusion of compounds during practical drug discovery applications. The traditional PAMPA system utilizes a hexadecane membrane or phospholipid-based barrier. The effective permeability (Pe) is calculated from the time-dependent change in compound concentration between donor and acceptor compartments, with standardized incubation periods typically ranging from 4 to 16 hours [13].

Lipid composition variations

Different lipid extracts have been employed to model specific biological barriers. For example, porcine brain polar lipid extracts are commonly used to model blood-brain barrier permeability, while intestinal-specific membranes can be created using different lipid combinations to better mimic gastrointestinal absorption.

Real-Time PAMPA (RT-PAMPA)

An innovative modification enabling direct fluorescence detection has been introduced, allowing real-time monitoring of drug permeation without sample transfer. This advancement addresses previous limitations by eliminating the need for post-incubation sample handling and enabling rapid differentiation between fast and slow diffusion events.

PAMPA's simplicity and non-biological nature provide significant benefits, particularly in the lead optimisation phase of drug discovery:

High throughput and automation

PAMPA is conducted in a 96-well format, allowing for the rapid and simultaneous screening of thousands of compounds. This makes it ideal for HTS and prioritizing chemical libraries.

Low cost and speed

The assay is significantly less expensive and faster than cell-based assays like Caco-2, typically requiring only 4–16 hours and avoiding the costly and time-consuming cell culture (21-day differentiation) and maintenance.

High reproducibility

As an abiotic system, it lacks the biological variability associated with cell lines (e.g., batch-to-batch or lab-to-lab variation in Caco-2 TEER), leading to highly reproducible data.

Application of PAPMA

Bioequivalence Assessment: PAMPA has been applied to evaluate bioequivalence of generic formulations. The MacroFlux system, a scaled-up version of PAMPA, successfully predicted in vivo bioequivalence risk by identifying formulations with significantly lower permeability than reference products. This application demonstrates PAMPA's utility as an auxiliary method for predicting bioequivalence and analysing factors responsible for bioequivalence risk [14].

Advanced computational and predictive approaches in PAMA

QSAR Modelling with PAMPA Data

Quantitative structure-activity relationship (QSAR) models have been successfully developed using PAMPA permeability data. Hierarchical support vector regression (HSVR) schemes demonstrate superior performance compared to partial least squares (PLS) for predicting PAMPA effective permeability, providing both predictive accuracy and mechanistic interpretability [15].

Franz Diffusion Cell

IVPT requires a diffusion cell with donor and receptor compartments separated by the skin; the topical formulation is applied to the donor compartment and the receptor compartment typically contains an aqueous medium. The vertical diffusion cell known as the Franz cell (Figure 2) was developed by Dr. Thomas Franz in 1975.

The core function of the Franz cell is to model the process of a substance moving from a drug formulation, through a membrane (like skin), and into a receiving medium. This movement is typically governed by the principle of passive diffusion, where molecules move from an area of high concentration (the drug formulation) to an area of low concentration (the receptor fluid) down a concentration gradient [16].

The Franz diffusion cell apparatus typically consists of two main sections separated by a membrane:

Donor compartment (Upper Chamber)

This is where the test sample (the topical drug formulation) is applied to the surface of the membrane.

It is often open to the air, allowing for application of a fixed or "finite" dose, mimicking real-world use.

Receptor compartment (lower chamber)

This is a jacketed glass vessel filled with a receptor fluid (or diffusion medium), such as a buffer solution, which simulates the body's subcutaneous tissue or blood flow.

It has an inlet and outlet for a circulating water bath to maintain the fluid at a constant, physiological temperature, usually 32°Cor 37°C. A small magnetic stirring bar continuously mixes the receptor fluid to ensure a uniform concentration and maintain "sink conditions"—meaning the concentration of the diffusing drug remains very low, which helps to keep the diffusion rate constant and similar to the body's continuous blood flow removing the drug [17].

Current challenges and future direction

Technical and methodological challenges in IVPT are primarily from the absence of standardised protocols across laboratories, creating substantial variability in experimental conditions, including temperature control, receptor fluid composition, and membrane preparation procedures. The selection of appropriate biological membranes represents a critical challenge, as animal tissues may inadequately reflect human skin physiology, and variations in tissue source, storage conditions, and barrier integrity significantly impacts permeation outcomes. Maintaining sink conditions another challenge for IVPT testing. Ensuring the concentration of the permeated drug in the receptor medium remains low enough (typically <10% of saturation) throughout the experiment to mimic the continuous drug clearance *in vivo* is often difficult, especially for highly lipophilic or slowly permeating drugs. Formulation-specific challenges emerge from the complexity of establishing meaningful correlations for semi-solid products, where excipient composition and vehicle microstructure critically influence drug permeation profiles. The sensitivity of IVPT methods to detect subtle formulation differences remains insufficient, as current approaches may fail to discriminate between products with minor compositional variations that could impact therapeutic outcomes. Regulatory acceptance barriers persist across different applications, particularly for topical bioequivalence assessment, where IVPT data alone may prove insufficient without supporting clinical endpoint studies or validated surrogate measures [18].

Figure 2. Franz diffusion cell model

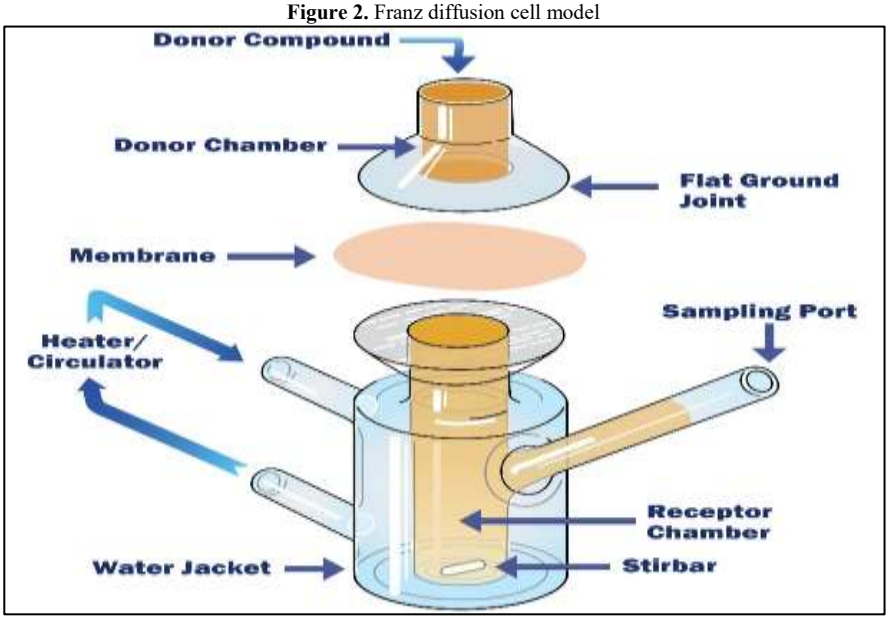


Table 2. Comparison between different route and its IVPT parameters

Application Area	Technical Limitations	Validation Requirements	Regulatory Hurdles
Dermatological	Membrane variability (animal vs. human tissue); inconsistent preparation protocols; temperature control variations	Demonstration of barrier integrity; permeation kinetics validation; IVIVC establishment for complex formulations	Q1/Q2/Q3 similarity required; IVPT + clinical endpoints for complex products; limited acceptance without pharmacodynamic data
Mucosal	Route-specific physiological differences (nasal, rectal, vaginal); mucus layer variability; pH and enzyme activity challenges	Tissue viability maintenance; receptor fluid optimization for each route; method sensitivity for low permeation drugs	No harmonized guidelines across mucosal routes; limited precedent for bioequivalence decisions; clinical studies often required
Ophthalmic	Complex ocular barriers (corneal, conjunctival); tear film dynamics; rapid clearance mechanisms	Drug retention assessment; permeation across multiple tissue layers; formulation-specific release kinetics	Limited IVPT acceptance for ophthalmic products; clinical trials remain primary requirement; in vivo residence time correlation challenges

Table 3. Differentiation between EMA and FDA regulatory guidelines on IVPT

Parameter/Acceptance Criteria	EMA 2018 [20]	FDA 2022 [21]
Duration of IVPT	24 hours.	Up to 48 hours.
Sampling frequency of the receptor compartment	Sampling points must be sufficient to capture the maximal rate of absorption and the subsequent decline in absorption rate.	The study duration must capture the maximum (peak) flux and its decline. A minimum of eight non-zero sampling time points is recommended.
Amount of sample in donor chamber	2-15 mg/cm2	5-15 mg/cm2
Number of skin donors and number of replicates per donor	A minimum of 12 skin donors with at least 2 replicates per donor is required.	The applicant is responsible for determining the number of donors to adequately power the pivotal study. A minimum of 4 dosed replicates per donor per treatment group (test or reference) is recommended.
Acceptance criteria for equivalence parameters, Jmax and A total for test and reference products	90 % C.I. for means of test and comparator ratios should lie between 80 – 125 % Wider 90 % C.I. limits to a max. of 69.84–143.19 % may be accepted for low strength and limited diffusion drug products and if clinically justified	Within-door variability for the reference product used to determine if Average Bioequivalence or Scaled Average Bioequivalence statistical analysis is used 90 % C.I. of ratio of test and comparator geometric means is 80 – 125 %

Advanced analytical methodologies act as a transformative frontier in IVPT technology, with Raman microspectroscopy emerging as a particularly promising technique for non-invasive, chemically-specific measurement of medicine penetration and permeation kinetics in situ. This optical technique has demonstrated capability to distinguish skin delivery from different formulations & provides real-time monitoring without tissue destruction.

The development of more sophisticated, physiologically relevant 3D skin models using bioprinting and induced pluripotent stem cells (iPSCs) that incorporate multiple cell types and features, such as vascularisation, is a key future direction to better mimic the native skin architecture and function. Microfluidic systems & organ-on-a-chip devices are emerging to create dynamic, highly controlled microenvironments that grant for *in situ* monitoring of drug permeation and can integrate aspects such as continuous fluid flow (simulating blood clearance). Integrating AI/ML with IVPT data for analysis and modelling can enhance the predictive power of the test, potentially leading to more accurate predictions of *in vivo* outcomes and a stronger foundation for IVIVC [19].

CONCLUSION

The in vitro permeation test (IVPT) has established itself as the definitive non-clinical methodology for evaluating drug delivery from complex topical and transdermal formulations. By utilising biological membranes and simulating physiological conditions, IVPT provides critical data on the rate and extent of drug penetration, distinguishing it from quality-focused tools such as the In Vitro

Release Test (IVRT). While various cell-based (Caco-2, MDCK) & non-cell-based models (PAMPA) offer valuable high-throughput screening capabilities, IVPT remains pivotal for demonstrating bioequivalence as it is increasingly required by regulatory agencies such as the FDA and EMA. Future advancements, including 3D bioprinted skin models, Raman microspectroscopy, and the integration of Artificial Intelligence (AI/ML), promise to increase the predictive power and sensitivity of these assays. Ultimately, the continued refinement and standardisation of IVPT protocols will be essential for accelerating the development of safe and effective generic topical drug products.

Conflict of interest

None

REFRENCE

1. Chang RK, Raw A, Lionberger R, et al, 2013. Generic development of topical dermatologic products: formulation development, process development, and testing of topical dermatologic products. The AAPS journal. 15(1), Pages 41-52. Doi: 10.1208/s12248-012-9411-0.

2. Santos LL, Swofford NJ, Santiago BG, 2020. In vitro permeation test (IVPT) for pharmacokinetic assessment of topical dermatological formulations. Current Protocols in Pharmacology. 91(1), Pages e79. Doi: 10.1002/cpph.79.

3. Hoppel M, Tabosa MA, Bunge AL, et al, 2021. Assessment of drug delivery kinetics to epidermal targets in vivo. The AAPS Journal. 23(3), Pages 49.

4. Shah VP, Miron DS, Rădulescu FȘ, et al, 2022. In vitro release test (IVRT): Principles and applications. International Journal of Pharmaceutics. 626, Pages 122159. Doi: 10.1016/j.ijpharm.2022.122159.

5. Brito S, Baek M, Bin BH, 2024. Skin structure, physiology, and pathology in topical and transdermal drug delivery. Pharmaceutics. 16(11), Pages 1403. Doi: 10.3390/pharmaceutics16111403.

6. Ali S, Shabbir M, Shahid N, 2015. The structure of skin and transdermal drug delivery system-a review. Research journal of pharmacy and technology. 8(2), Pages 103-109. Doi: 10.5958/0974-360X.2015.00018.7.

7. Souto EB, Fangueiro JF, Fernandes AR, et al, 2022. Physicochemical and biopharmaceutical aspects influencing skin permeation and role of SLN and NLC for skin drug delivery. Heliyon. 8(2). Doi: 10.1016/j.heliyon. 2022.e08938.

8. Kus M, Ibragimow I, Piotrowska-Kempisty H, 2023. Caco-2 cell line standardization with pharmaceutical requirements and in vitro model suitability for permeability assays. Pharmaceutics. 15(11), Pages 2523. Doi: 10.3390/pharmaceutics15112523.

9. da Silva VD, Canales CS, Polinário G, et al, 2025. Advancements in techniques for analysing cell permeability. Heliyon. 11(14).

10. Irvine JD, Takahashi L, Lockhart K, et al, 1999. MDCK (Madin–Darby canine kidney) cells: a tool for membrane permeability screening. Journal of pharmaceutical sciences. 88(1), Pages 28-33. Doi: 10.1021/js9803205.

11. Hansson GC, Simons K, van Meer G, 1986. Two strains of the Madin-Darby canine kidney (MDCK) cell line have distinct glycosphingolipid compositions. The EMBO Journal`. 5(3), Pages 483-489. Doi: 10.1002/j.1460-2075.1986.tb04236.x.

12. Masungi C, Mensch J, Van Dijck A, et al, 2008. Parallel artificial membrane permeability assay (PAMPA) combined with a 10-day multiscreen Caco-2 cell culture as a tool for assessing new drug candidates. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 63(3), Pages 194-199.

13. Behrens I, Kamm W, Dantzig AH, et al, 2004. Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assays in drug discovery. Journal of pharmaceutical sciences. 93(6), Pages 1440-1453. Doi: 10.1002/jps.20062.

14. Chou J, Lai R, Chou J, et al, 2023. Enhancing Bioequivalence Predictions with PAMPA Dissolution Using Formulations of Telmisartan. Journal Name (implied). Pages 109-114.

15. Chi CT, Lee MH, Weng CF, et al, 2019. In silico prediction of PAMPA effective permeability using a two-QSAR approach. International journal of molecular sciences. 20(13), Pages 3170. Doi: 10.3390/ijms20133170.

16. Kumar M, Sharma A, Mahmood S, et al, 2024. Franz diffusion cell and its implication in skin permeation studies. Journal of Dispersion Science and Technology. 45(5), Pages 943-956.

17. Ng SF, Rouse JJ, Sanderson FD, et al, 2010. Validation of a static Franz diffusion cell system for in vitro permeation studies. Aaps Pharmscitech. 11(3), Pages 1432-1441. Doi: 10.1208/s12249-010-9522-9.

18. Alomari N, Alhussaini W, 2024. Update on the advances and challenges in bioequivalence testing methods for complex topical generic products. Frontiers in Pharmacology. 15, Pages 1330712. Doi: 10.3389/fphar.2024.1330712.

19. Javaid M, Haleem A, 2021. 3D bioprinting applications for the printing of skin: A brief study. Sensors International. 2, Pages 100123. Doi: 10.1016/j.sintl.2021.100123.

20. García-Arieta A, Gordon J, Gwaza L, et al, 2023. Regulatory requirements for the development of second-entry semisolid topical products in the European union. Pharmaceutics. 15(2), Pages 601. Doi: 10.3390/pharmaceutics15020601.

21. Muhammad Adil, Faten Zubair Filimban, Ambrin, Atifa Quddoos, 2024. Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of Euphorbia parviflora L. (Euphorbiaceae Juss.). Scientific Reports. 14, Pages 5627. Doi: 10.1038/s41598-024-55905-w

22. Sreyly Heng, Tatsuya Nakayama, Waraporn Kusalaruk, 2025. Antibacterial activity of ethanol plant extract combined with organic acid against pathogenic and spoilage bacteria at different pH conditions. Discover Food. 5, Pages 399. Doi: 10.1007/s44187-025-00616-y.