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***In vitro* permeation testing for the evaluation of drug delivery to the skin**

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Abstract

This review considers the role of *in vitro* permeation testing (IVPT) for the evaluation of drug delivery from topical formulations applied to the skin. The technique was pioneered by Franz in the 1970's and today remains an important tool in the development, testing and optimization of such topical formulations. An overview of IVPT as well as selection of skin for the experiment, integrity testing of the membrane, and required number of replicate skin samples is discussed. In the literature many researchers have focused solely on permeation and have not reported amounts of the active remaining on and in the skin at the end of the IVPT. Therefore, a particular focus of this article is determination of the complete mass balance of the drug. It is noteworthy that for the evaluation of bioequivalence of topical formulations the draft guideline issued by the European Medicines Agency (EMA) requires the IVPT method to report on both the skin deposition and distribution of the active in the skin as well as amount permeated. Other aspects of current guidance from the EMA and United States Food and Drug Agency for IVPT are also compared and contrasted. Ultimately, harmonisation of IVPT protocols across the regulatory agencies will expedite the development process for novel topical formulations as well as the availability of generic products.

Key words: *In vitro* permeation testing, Franz cell, skin, mass balance, EMA, FDA

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1. Introduction

The methods currently used to study the dermatopharmacokinetics of drug substances may be broadly classified as *in vitro* models and *in vivo* studies. Considering the former category, results from *in vitro* permeation testing (IVPT) using human skin (Franz et al., 2009; Lehman et al., 2011) have been shown to correlate with clinical studies of skin uptake and bioequivalence. Clearly, IVPT with human skin offers significant advantages, given that *in vivo* human studies are expensive to perform, time consuming and they have safety or ethical complications. In this review we consider the main parameters for design and conduct of IVPT including cell design, dosing of formulations, duration of the study and sampling, selection and preparation of skin and number of skin samples and replicate experiments. The topic of mass balance is given particular attention as, over the years, many reports in the literature have focused solely on assessment of permeation of the active in IVPT. However, a well conducted experiment allows reporting of the total drug disposition following topical application in IVPT, including drug deposition on the skin, retention in skin as well as full penetration through skin.

To date, protocols for conducting IVPT have been published by a number of regulatory agencies or organisations including the Organisation for Economic Co-operation and Development (OECD), the Scientific Committee on Consumer Safety (SCCS), European Medicines Agency (EMA) and the United States Food and Drug Administration (OECD 2004b,c, 2011 ; SCCS 2010; EMA, 2018; FDA, 2022). However, the recommendations for IVPT outlined in the EMA and FDA guidelines are focused on the application of this method for demonstration of bioequivalence of a topical formulation compared with a reference drug product. Interestingly, the EMA guideline notes that while the document addresses locally applied and locally acting medicinal products for cutaneous use, it is also relevant for other medicines e.g. preparations for auricular or ocular use. The FDA guidance states that is applicable to “liquid-based and/or other semisolid products applied to the skin including integumentary and mucosal (e.g., vaginal) membranes”. Thus, specific requirements of the EMA and FDA guideline for IVPT will also be considered and those that should be further explored for harmonization from a regulatory perspective are identified.

2. IVPT – historical development and diffusion cell designs

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 early papers that reported the application of diffusion cells and other methods to evaluate skin permeability of actives have been reviewed by Tregear (1966) and Ainsworth (1960). These researchers utilized a horizontal diffusion cell design that is rarely used today and will not be discussed further. The vertical diffusion cell known as the Franz cell (Figure 1) was

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developed by Dr. Thomas Franz in 1975 (Franz, 1975). This glass cell was specifically designed to examine *in vitro* percutaneous absorption and to determine how the data generated might be correlated with *in vivo* results. In the original study with the Franz cell the membrane was held between two ball joints on an O-ring with a joint clamp. Later, Anjo and colleagues (1980) and Cooper (1982) devised diffusion cell arrays for use in an automated fraction collector. A flow-through Teflon diffusion cell apparatus was evaluated by Bronaugh and Stewart (1985). The cell array/flow-through cell design offers the advantage of automation of sample collection in contrast to the manual sampling required for the stand alone cell (Figure 2). Most recently Sil et al. (2018) reported the production of 3D printed Franz cells fabricated with resin using computer aided design and stereolithography 3D printers (Figure 3). The basic Franz cell design has also been modified by various workers to study permeation through excised nail and scalp tissues (Mertin and Lippold, 1997; Paz-Alvarez, 2019).

3. Conducting the permeation experiment

For all cell designs, in order to perform a permeation test the skin to be used is mounted as a barrier between the donor and receptor compartments. Membrane integrity should be assessed prior to commencement of the experiment. Accurate measurement of cell dimensions is important for subsequent data analysis. The skin is allowed to equilibrate so that the temperature of the skin surface is at 32°C prior to commencing the permeation experiment. Typically, the temperature will be maintained using either a heating block, immersing cells in a water bath or using jacketed cells connected to a water source for temperature control. It is good practice to monitor the surface skin temperature over the course of the experiment. The importance of temperature control for IVPT cannot be overstated. A study by Chilcott et al (2005) investigated inter- and intralaboratory variation in diffusion cell measurements attributed to factors other than skin for 18 different laboratories. Permeation of a test substance was determined in an artificial membrane with average flux values reported for each laboratory. The value for the coefficient of variation between laboratories was reported as 35%. One of the factors contributing to this observed variation was suggested to be the actual membrane temperature rather than the nominal diffusion cell temperature.

The amount of formulation applied for finite dose conditions typically ranges from 2 to 10 μl/cm^2 and the donor chamber is not occluded. Selection of finite versus infinite dose application of formulations as well as the choice of receptor medium is discussed further below. Throughout the experiment the receptor medium is agitated, usually with a magnetic stirring bar for the stand-alone cell. For the flow-through cell this is achieved by continuous replacement of the receptor medium. During sampling it is important that air bubbles are not introduced underneath the skin. The duration of the experiment will depend on the number of sampling points required and the purpose of the test.

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At the end of the experiment a profile of cumulative absorption of drug per unit area of skin over time should be constructed (Figure 4). Although mathematical expressions for data analysis of infinite dose permeation profiles are relatively straightforward (Mitragotri et al., 2011) the approaches used for finite dose permeation studies are more complex (Santos et al., 2012).

4. Receptor compartment and medium

The composition of the receptor medium must be selected so that it simulates the scenario observed during *in vivo* permeation of an active ingredient, where the permeant is constantly cleared by the blood flow. The receptor solution has to provide and maintain throughout the experiment "sink" conditions so that the absorption of the active ingredient is not hindered (OECD, 2004a,b). This is achieved by ensuring that the thermodynamic activity of the active in the receptor solution does not exceed 10% of its thermodynamic activity in the donor solution (Skelly et al., 1987). While achieving sink conditions may not be a problem in the flow-through apparatus, for the stand-alone cell optimisation of sample collection times or sample volume may be required for poorly soluble actives. Where the active has adequate water solubility phosphate buffered saline (PBS), pH 7.4 is considered an acceptable medium that replicates the physiological environment. If sink conditions cannot be maintained for the active in PBS, then various additives or alternatives to PBS may be added to address this problem. To this end, some researchers have used bovine serum albumin (Cross et al., 2003) as the protein reversibly binds to the active. The use of the non-ionic surfactant polyethylene glycol (PEG)-20-oleyl ether (Brij™, Volpo™) is also reported (Song et al., 1998; Santos et al., 2010). Although ethanol and isopropanol have also been used as components of the receptor phase for IVPT there is evidence that ethanol may compromise the skin barrier integrity (Ramsey et al., 1990; Sarnthiratch et al., 2024). Interestingly the recent FDA draft guidance related to the use of IVPT (FDA, 2022) notes "…inclusion of organic solvents and alcohols in the receptor solution… are not recommended and may invalidate the IVPT method". Where a preservative is added to the receptor medium it should also have no effects on the tissue integrity. To avoid the presence of air bubbles on the tissue it is also good practice to deaerate the medium. Finally, the receptor phase should not affect the stability of the active ingredient.

5. Sampling volume, sampling intervals and experiment duration

Two experimental parameters which are closely related to the concept of sink conditions are the sampling volume and the sampling intervals. The volume of the sample should be large enough to allow analysis of the active and should always be replaced with an equal volume of fresh receptor solution to maintain sink conditions. An excessively large sampling volume may cause an unnecessary

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dilution of the receptor solution and affect the quantification of the active in samples collected at later time points. Conversely, collection of very small sample volumes may lead to accumulation of the active in the receptor solution and loss of sink conditions. The frequency of sampling intervals should allow the determination of the absorption profile of the test substance (OECD, 2004a,b). If too many samples are collected, the active may be diluted and quantification may be affected. If the sample collection is not frequent enough, the active may accumulate in the receptor solution causing loss of sink conditions. It is thus clear that a balance between sampling volume and sampling intervals has to be established. To further complicate the situation, the absorption rate of the active ingredient also plays a role and affects both of the above parameters. Given the intrinsic difficulty in establishing these variables *a priori*, pilot experiments should be conducted, especially for active ingredients with unknown permeation characteristics. Ideally, the duration of the permeation experiments should be determined based on to the absorption rate of the active ingredient (OECD, 2004a,b). An experiment performed with an active that permeates rapidly may be conducted for a shorter period of time, depending on the skin target site. Although most topical formulations are dosed on a daily basis some permeation experiments have been conducted up to 48 h; where the goal is transdermal delivery of the drug experiments may extend further (Wang et al., 1990; Roy et al., 1996). Confirmation of skin integrity should be ensured where such prolonged studies are performed (Howes, 1996).

6. Mass balance studies, selection of skin washing and skin extraction solvents

OECD guideline 428 (OECD 2004a) notes the importance of mass balance determination when conducting skin absorption studies. Specifically, the guidelines states that all components of the test system should be analysed and recovery is to be determined. This will require removal of the skin from the cells, washing of the surface, extraction of any active in the skin to give the total sum of active recovered from permeation, skin uptake and surface deposition. The total recovery value required by the guideline is 100±10% of the amount of active applied. OECD GD28 (OECD 2004b) contains the same recommendation, with a caveat that for volatile test substances and unlabelled test substances, a range of 80–120% is acceptable. The EMA draft guideline (2018) also notes that the mass balance of the drug should be determined; an acceptable value for overall recovery of the drug is recommended as 90-110%.

In order to perform a successful mass balance study, the solvents used to wash the skin surface and to extract the skin membrane must be carefully selected. For the skin surface washing, a solvent which can dissolve both the vehicle of the formulation and large amounts of the active ingredient should be chosen. Similar considerations dictate the selection of the extraction solvent. In this case, the solvent should not only be able to solubilise a considerable amounts of the active ingredient but

the solvent itself should easily penetrate the skin to extract the active efficiently. For example, Parisi et al. (2016) selected water and dimethyl sulphoxide (DMSO) for mass balance studies with hydrophilic and hydrophobic vehicles, respectively, following IVPT with two hexamidine salts. As the authors had also confirmed good solubility of the actives in DMSO and methanol and both solvents are known to easily penetrate the skin (Dutkiewicz et al., 1980; Jacob and Herschler, 1986), a 50:50 mixture of the two was used for skin extraction. It is necessary to validate the mass balance method with the solvents selected for washing and extraction. The stability of the active ingredient in the solvents used for the skin washing and extraction procedures should also be verified.

7. Dose selection

The dose applied to the donor compartment is a critical factor in the design of an *in vitro* permeation experiment and subsequent mass balance studies. Depending on the amount and the concentration of the applied formulation, two scenarios are possible (Franz et al., 1993). If the amount of applied formulation is sufficiently large so that neither the active nor the vehicle is considerably reduced over time, the dose is considered infinite and a characteristic steady-state profile is observed (Figure 4). If only a very small amount (e.g. ≤ 10 μl or mg/cm^2) of formulation is applied and a substantial reduction of the active and/or the vehicle is observed, the dose is considered finite. The selection of the type of dose is primarily determined by the objective of the study. For example, an infinite dose is usually preferred when investigating the permeation enhancement effect of solvents and their interactions with the membrane or when evaluating the permeation of actives which have not been studied before. The principal disadvantage of using an infinite dose is that it is not representative of the *in vivo* situation.

A finite dose is appropriate when a clinically relevant application, which mimics as close as possible the *in vivo* situation, is required. A further issue that has to be considered is that infinite and finite doses generally produce very different rates and total amounts of absorption (Franz et al., 1993; Wester et al., 1998a; Oliveira et al., 2012). It will be evident that finite doses are appropriate for the evaluation of topical bioequivalence with reference to the majority of semi-solid dosage forms. Figure 4 illustrates a schematic of finite dose application and the expected non-linear permeation profile where dose depletion occurs. The draft EMA guideline states that the recommended dosing amount should be in the rage of 2-15mg/cm^2 and the donor compartment should not be occluded. Dose application should be validated to ensure reproducibility (±5 %) and homogeneous spreading of the formulation over the skin membrane (EMA, 2018). In an earlier FDA draft guidance for demonstration of bioequivalence of topical acyclovir cream using IVPT the dose recommended was a single, un-occluded dose in the range of 5-15 mg cream/cm^2 (FDA, 2016). The most recent guideline (FDA, 2022)

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recommends that the selection of the dose amount used in the study should be assessed for each IVPT method based upon studies performed during IVPT method development.

8. Tissue selection and preparation for permeation studies

Over the years human and animal tissues have been used in skin diffusion cell experiments. Clearly, the “gold standard” is human skin but this may be difficult to source and there may be ethical issues associated with acquisition of the tissue. Typically, human skin may be obtained from cadavers or following plastic surgery procedures. Depending on the purpose of the permeation study fresh or frozen skin may be used. Where the compound of interest requires skin metabolism and viability fresh skin is necessary (Fahmy et al., 1993; Wester et al., 1998b). Several authors have confirmed that the integrity of human skin is not reported to be compromised following freezing (Harrison et al., 1984; Kasting and Bowman, 1990; Barbero and Frasch, 2016). Various methods may be used to prepare the skin prior to mounting in the diffusion cell apparatus. Kligman and Christophers (1963) first reported the heat separation method for preparation of human epidermal skin membranes. Essentially full thickness skin is exposed to a temperature of 60°C in a water bath for two to three minutes. After removal from the water, the epidermal sheet is carefully peeled off and mounted on a filter paper support. Lower temperatures and shorter exposure periods have also been reported to effectively weaken the adhesion between the epidermis and dermis (Netzlaff et al., 2006; Atrux-Tallau et al., 2007; Oliveira et al., 2012). Human skin samples may also be collected using a dermatome. This device employs a sharp blade to cut a piece of skin; sample thickness is controlled by a calibrated instrument setting (Bronaugh and Collier, 1991). Dermatomed skin samples are thicker than epidermal membranes and retain a portion of the dermis. It is also possible to mount full thickness human skin samples comprising SC, epidermis and dermis in diffusion cells. The underlying subcutaneous tissue must be removed from the skin prior to use.

For bioequivalence determination the EMA guideline requires the use of human skin. The guideline further requires that the IVPT study protocol should specify the inclusion/exclusion criteria for skin sections, the anatomical region, condition, and duration of skin storage. Skin with tattoos, any signs of dermatological abnormality or exhibiting a significant density of terminal hair should be excluded. Different skin preparations may be used and it is further recommended that skin from different donors should be chosen (EMA, 2018). The FDA acyclovir guidance stated that excised human skin with a competent skin barrier should be used (FDA, 2016). The more recent IVPT guidelines also recommends excised human as the membrane for the IVPT study. The guidance further notes that information should be provided to briefly explain the choice of skin source (e.g., cadaver), skin type (e.g., posterior torso) and skin preparation for example, dermatomed skin (FDA, 2022).

9. Integrity testing of tissue

As skin samples may be damaged during collection, preparation, handling and storage it is important to ensure skin integrity is confirmed prior to conducting the permeation experiment. The methods currently used include assessment of tritiated water permeability, measurement of transepidermal water loss (TEWL), and determination of transcutaneous electrical resistance (Fasano et al., 2002; Davies et al., 2004; Zhang et al., 2018). TEWL is specifically mentioned as a method that may be used for this purpose in the EMA quality and equivalence guideline (EMA 2018). Depending on the research group and specific method to confirm skin barrier integrity the values or criteria set for rejection of skin samples have varied (White et al., 2011). The EMA guideline notes that the skin integrity should be checked prior to and after each IVPT experiment. This is an interesting requirement as, following application of the test sample, it is expected that absorption and uptake of the active and vehicle components will inevitably change the barrier properties of the tissue. In addition, the guideline states that the choice of the skin integrity test and its acceptance criteria should be explained. Different acceptance criteria may be proposed for before and after the experiment; these acceptance criteria should be justified and consistent across all parallel experiments (EMA, 2018). In contrast, the three common methods for skin integrity testing are discussed in detail in the FDA guidance. For the TEWL method, information on number of replicate measurements per skin piece is provided. A value of 15 grams of water per square meter per hour (i.e., ≤ 15 $g/m^2/hr$) is mentioned as a reasonable skin barrier integrity acceptance (cutoff) criterion for a TEWL value of barrier integrity. The importance of optimising the TEWL measurement methods as part of IVPT is underlined as is the need to calibrate the TEWL device. Monitoring and reporting of the ambient laboratory temperature and humidity is also required (FDA, 2022).

10. Intra- and intervariability of human skin - implications for number of replicate experiments

Intra- and intersample variability in skin permeation is an important consideration when designing an *in vitro* permeation test with human skin. Various authors have studied in vitro datasets of skin permeability including Southwell and colleagues (1984), Kasting et al. (1994), Williams et al. (1992) and Cornwell and Barry (1995). Using a range of model compounds with excised abdominal skin, Southwell et al. (1984) reported the overall mean *in vitro* estimates of intersample variation in skin permeability as 66% ± S.D. 25 (n = 45) and intrasample variation as 43% ± 25 (n = 32). The authors also suggested that abdominal skin appeared to be less variable in its permeability than other anatomical sites. A large retrospective study of *in vitro* skin permeability data for breast and abdominal samples was reported by Meidan and Roper (2008). The values for tritiated water permeability

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coefficients for 2400 skin samples that were derived from 112 donors were analysed. For inter- and intraindividual comparisons smaller data subsets (15 donors) were also analysed. Interestingly, there was no correlation between age and skin permeability. The interindividual variability for the 112 donors followed a normal distribution. Overall, interindividual differences in skin barrier function (CV = 37.6%) were smaller than intra-individual site-dependent differences (38.3% 6 CV 6 115.7%).

Because of this reported variability in excised skin samples the number of replicate experiments to be conducted and the number of donors used has been the subject of much interest to researchers and regulatory authorities. Skelly et al. (1987) acknowledged this issue and recommended that when comparing two formulations 12 experiments should be run. However, no justification was provided for this specific recommendation. Mitra and colleagues (2016) used IVPT to demonstrate the equivalence of two butenafine hydrochloride formulations. In this study, the permeation test was validated using a pilot study with three formulations of different strengths and skin from one donor. For the definitive in vitro permeation study, a total of 24 abdominal skin samples (12 skin samples, with at least 2 replicates from each donor, for each formulation) from five donors were used. It is noted that a pilot IVPT study may be useful in determining sample size. The EMA guideline states that inter-subject or inter-donor variability should be minimised by a crossover study design; a pilot IVPT study may be useful in determining sample size. The guideline further notes that the number of skin donors should not be less than 12, with at least 2 replicates per donor; the number of donors may be less than 12 if justified (EMA, 2018). The most recent OECD Guideline Guidance notes on dermal absorption studies (OECD, 2022) states that at least 8 replicates from at least 4 different donors (interpreted in most countries to mean at least 2 replicates from each donor) should be used for permeation testing. In the FDA draft guidance on IVPT it is stated that a pilot IVPT study be performed with multiple skin donors (e.g., 4–6 skin donors); a minimum of four replicate skin sections per donor per treatment group is also recommended. Interestingly, for the pivotal IVPT study it is noted that it is the responsibility of the applicant to determine the number of donors required for adequate statistical power. A minimum of four dosed replicates per donor per treatment group (test product or reference product) is recommended as for the pilot study (FDA, 2022).

11. IVPT and regulatory requirements for bioequivalence

As noted above, both the EMA and FDA have issued guidelines on IVPT and how it may be applied to demonstrate bioequivalence of topical skin formulations compared with a reference listed product. From a review of both guidelines it will be evident that there are a number of differences in recommended parameters for the IVPT itself as well as the equivalence acceptance criteria. These differences are summarised in Table 1. The dissimilar requirements for number of skin replicates and

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donors deserve further consideration by the regulatory authorities. A particular concern will be the cost and availability of human tissues in different European countries compared with the USA. It is interesting that porcine skin was not referenced by either agency; many authors have reported on the close similarities between porcine and human tissues (Sato et al., 1991; Dick and Scott, 1992; Schmook et al., 2001; Barbero and Frasch, 2009). This may of course, reflect a reluctance to use animal tissues going forward, as is the case in the personal care sector but this has not been clarified.

For presentation of data the EMA guideline states that the relevant permeation parameters, are the maximal rate of absorption (Jmax) and total amount permeated at the end of experiment (Atotal). Similarly, FDA notes that the flux and cumulative permeation results from the IVPT pilot study and the eventual IVPT pivotal study should be calculated and reported. A bioequivalence limit of 80–125% is required by the EMA. Wider 90% confidence interval limits, to a maximum of 69.84 – 143.19 may be accepted in the case of high variability observed with low strength and limited diffusion drug products, and if clinically justified (EMA, 2018). In contrast, for the US-FDA, the within-reference variability is used to determine, if average bioequivalence or scaled average bioequivalence analysis should be is applied. Detailed guidance and numerical examples using simulated data sets illustrating the determination of bioequivalence are also outlined in the recent US FDA guideline (US FDA, 2022). These differences in the analysis and acceptance criteria for bioequivalence are deserving of wider debate but again, in part, reflect the different requirements for number of skin samples and replicates.

Compared with the FDA guidance a number of additional parameters are also required to be reported for the EMA. These additional permeation parameters include the time of maximal rate of absorption (tmax) and lag-times. It is further noted that the lag-times between the test and comparator products should be the same (i.e. within ± 10%) if present. As noted earlier, a mass balance determination is also necessary for the EMA guideline. The mass balance should be determined. As well as the cumulative amount of the active substance permeated into the receptor medium (Atotal), the total amount of active substance retained (Stotal) in the skin samples and amount of active substance retained on the cleaning or experimental equipment (Rtotal)should be presented. Finally, acceptance criteria for test and negative controls are addressed in the EMA guideline with a statement that the 90% confidence interval for the ratio of means of the test and negative control products should be entirely outside the interval of 80.00- 125.00%. Furthermore, the 90% confidence interval for the ratio of means of the comparator and negative control products should be entirely outside the interval of 80.00- 125.00% (EMA, 2018).

Since publication the draft EMA guideline has attracted considerable interest from scientists working in the field. A number of aspects of the guidelines have been the focus of recent publications by Miranda and co-authors (2020; 2022). The authors firstly reported the characterisation of eight

"blockbuster" topical formulations (Miranda et al., 2022). A number of batches of each formulation was selected with products being sourced from different manufacturing sites where possible. The product microstructure was assessed following the EMA guideline requirements and performance was also evaluated using in vitro release testing. Statistical analysis confirmed that none of the same product batches could be considered equivalent if EMA criteria were applied. Building on this work, the authors then evaluated a simple gel formulation and a more complex emulgel product with reference to rheology, IVRT and IVPT (Miranda et al., 2022). Again, equivalence could not be established with reference to rheological characterisation; IVPT equivalence could also not be demonstrated. Importantly, these studies have drawn attention to the difficulties companies will face if attempting to demonstrate bioequivalence following the current draft EMA guideline.

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Conclusions

As we approach 50 years since the seminal publication of Franz, it is clear that IVPT is firmly embedded as an important development tool for scientists working in a diverse array of disciplines. At the same time the various regulatory agencies in the personal care, pharmaceutical and environmental health sectors have appreciated the power and sensitivity of IVPT. The OECD was the first organisation to articulate protocols and guidance for IVPT; this paved the way for others such as the SCCS and the EMA to refine and develop their own guidelines. Most recently, detailed and comprehensive guidance has issued from the US FDA concerning IVPT. It is also important to note that various funding initiatives by the FDA have stimulated further research in IVPT, specifically how to harness the technique to develop efficient bioequivalence approaches for complex generic products. Ideally, requirements for IVPT would be harmonised across the different agencies and jurisdictions. An interlaboratory study with IVPT and human skin, similar to that reported for an artificial membrane should also prove insightful concerning further obstacles to uniform implementation of IVPT worldwide. A major challenge to effective and efficient use of IVPT is the difficulty associated with sourcing human tissues in different regions. Further discussion concerning the use of alternatives to human skin is needed particularly given the extensive literature reporting the similarities of human and porcine membranes. Ultimately, consensus on IVPT across the regulatory bodies and the scientific community will facilitate both novel topical medicines development as well as the accessibility of generic medicines.

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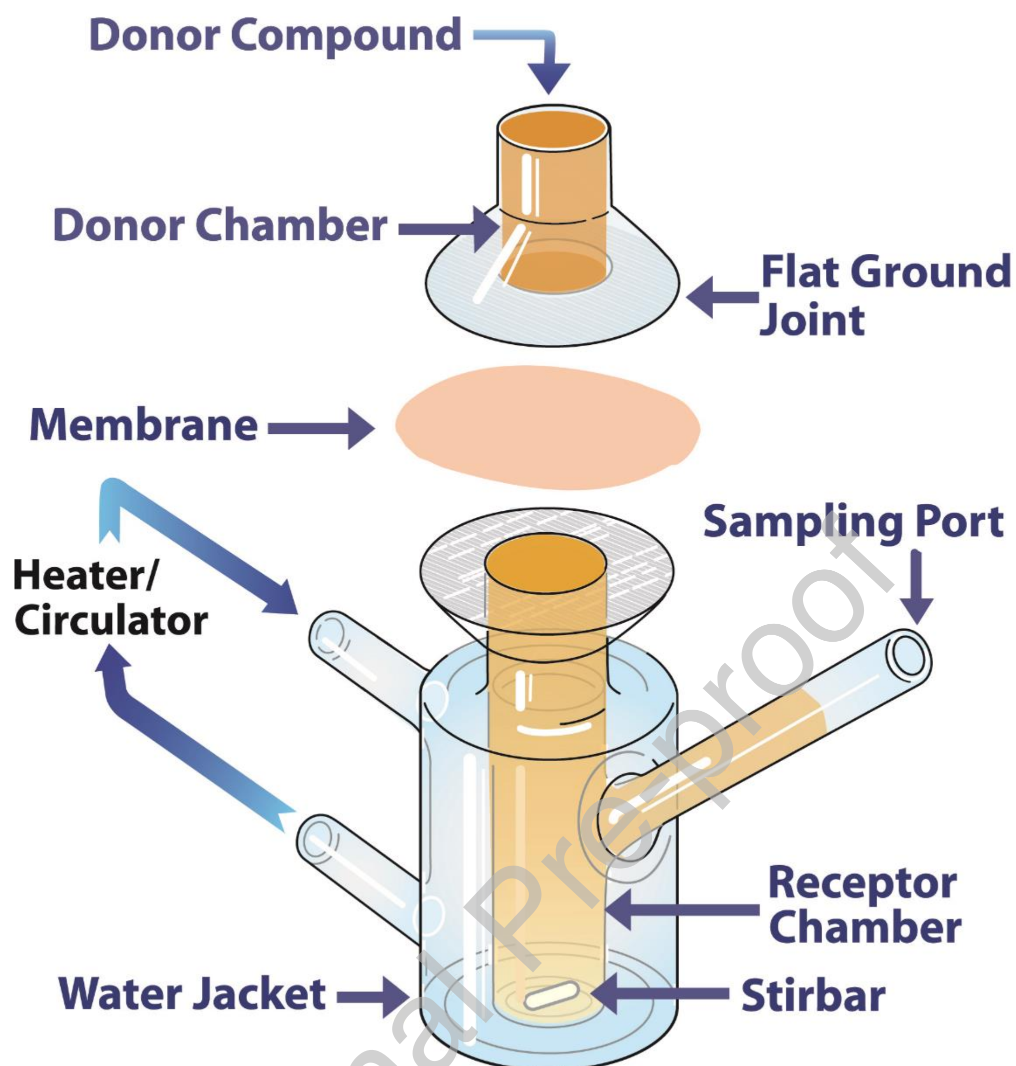

FIGURE 1: FRANZ DIFFUSION CELL. IMAGE COURTESTY OF PERMEGEAR

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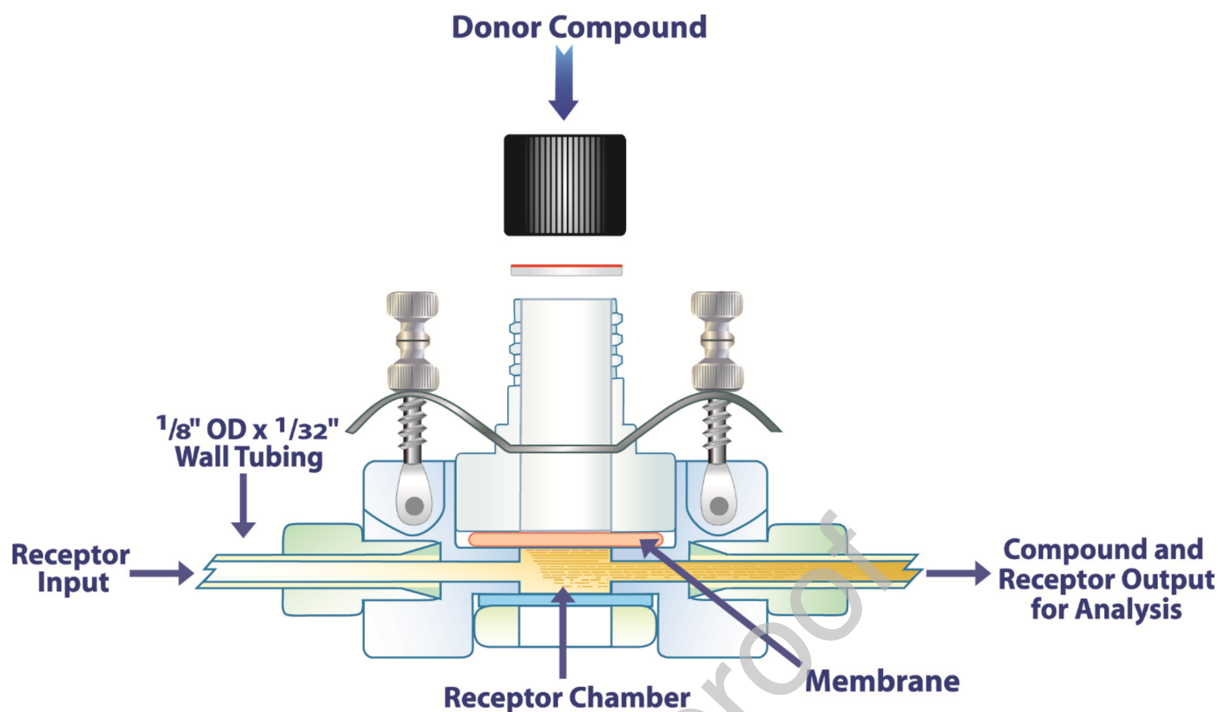

FIGURE 2: FLOW-THROUGH DIFFUSION CELL. IMAGE COURTESY OF PERMEGEAR.

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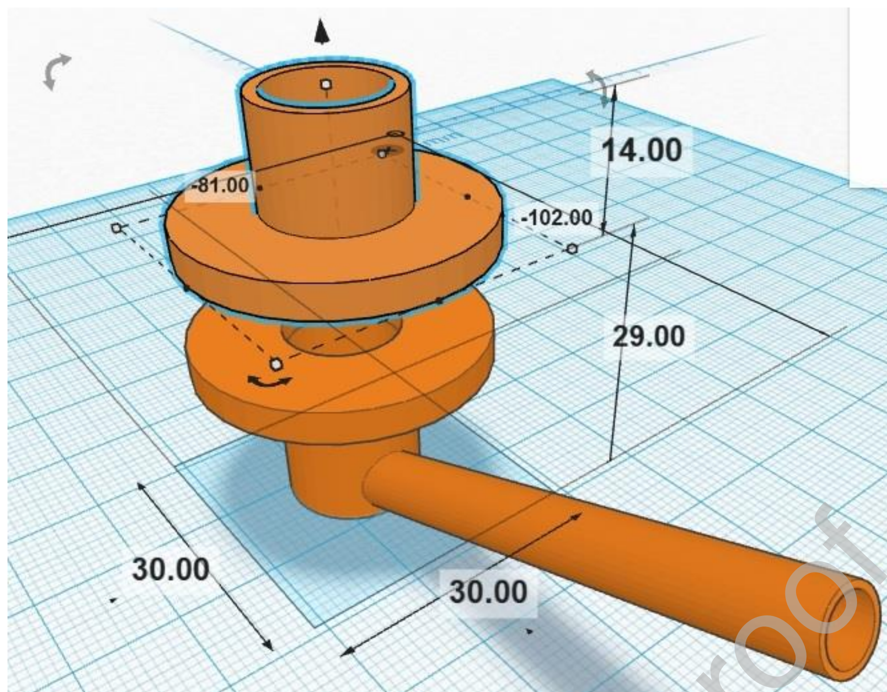

FIGURE 3: 3-D PRINTED FRANZ DIFFUSION CELL

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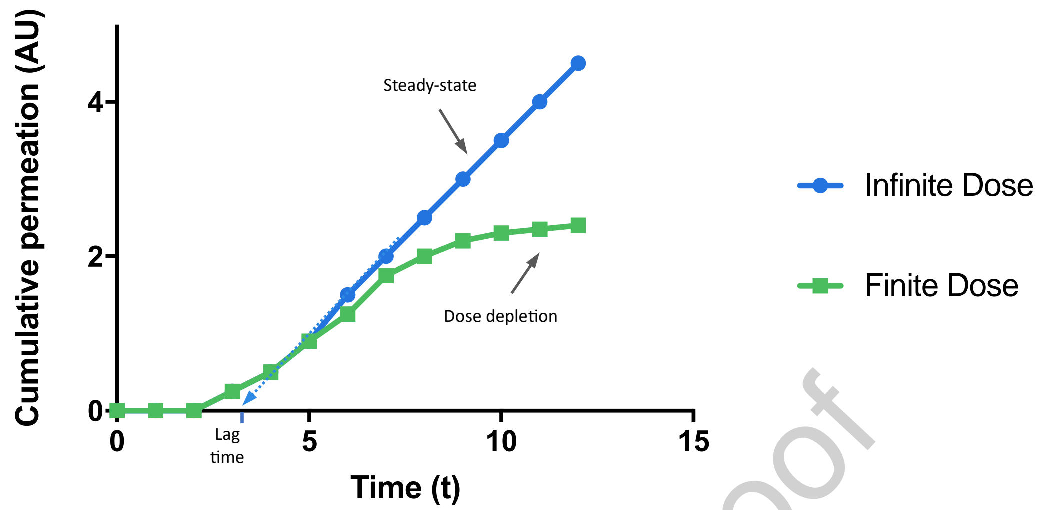

FIGURE 4: INFINITE VERSUS FINITE DOSE PERMEATION PROFILES. DOTTED LINE INDICATES INTERCEPT TO DETERMINE LAG TIME

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TABLE 1: COMPARISON OF EMA AND FDA GUIDELINES FOR IVPT

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Table 1: Comparison of EMA and FDA guidelines for IVPT

Parameter/Acceptance criteria	EMA 2018	FDA 2022
Duration of IVPT	24 h	Can be up to 48 h
Sampling frequency of receptor compartment	Number of sampling points should capture… maximal rate of absorption and decline in rate of absorption	Study duration should capture maximum (peak) flux and decline in flux… A minimum of eight non-zero sampling time points is recommended
Amount of sample in donor chamber	2 – 15 mg/cm^2	5 - 15 mg/cm^2
Number of skin donors and number of replicates per donor	Number of skin donors should not be less than 12 with at least 2 replicates per donor	Responsibility of applicant to determine number of donors to power pivotal study…..A minimum of 4 dosed replicates per donor per treatment group (test or reference) is recommended
Acceptance criteria for equivalence parameters, J_{max} 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 - 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%
Reporting of additional parameters	Lag times (±10% test and reference), time for max. permeation, full mass balance, C.I. limits for negative control and comparator products	Drug recovery reported as amount permeated into receptor chamber, dose depletion (not accounting for skin content) to be estimated and reported

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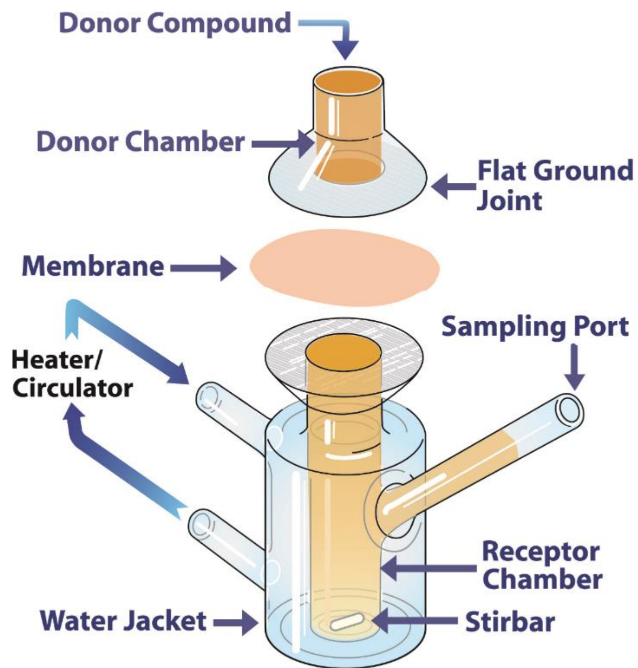
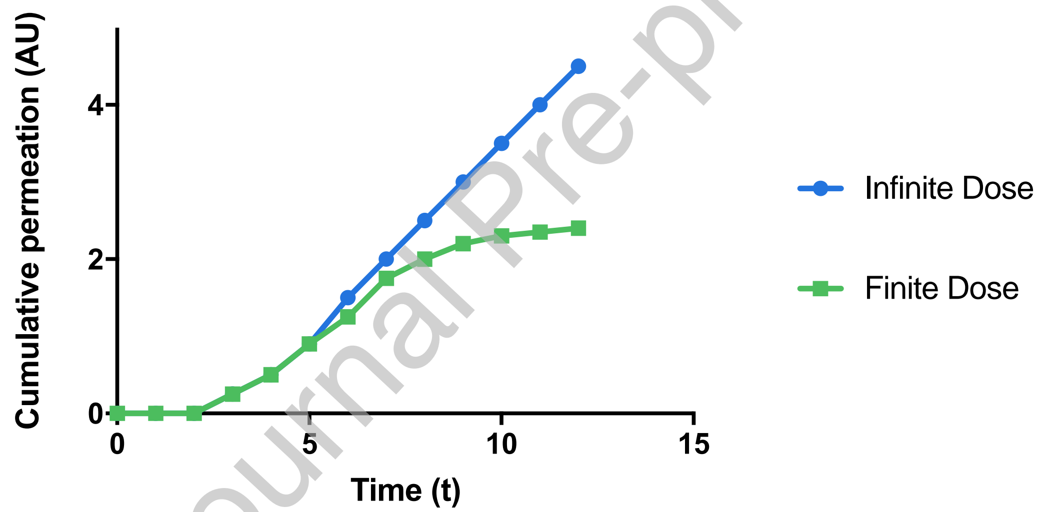
Graphical abstract





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