Estimation of in-vivo percutaneous permeation (flux) and cumulative amount input of metronidazole formulations in mini-pigs' dermis

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FDA Disclaimer

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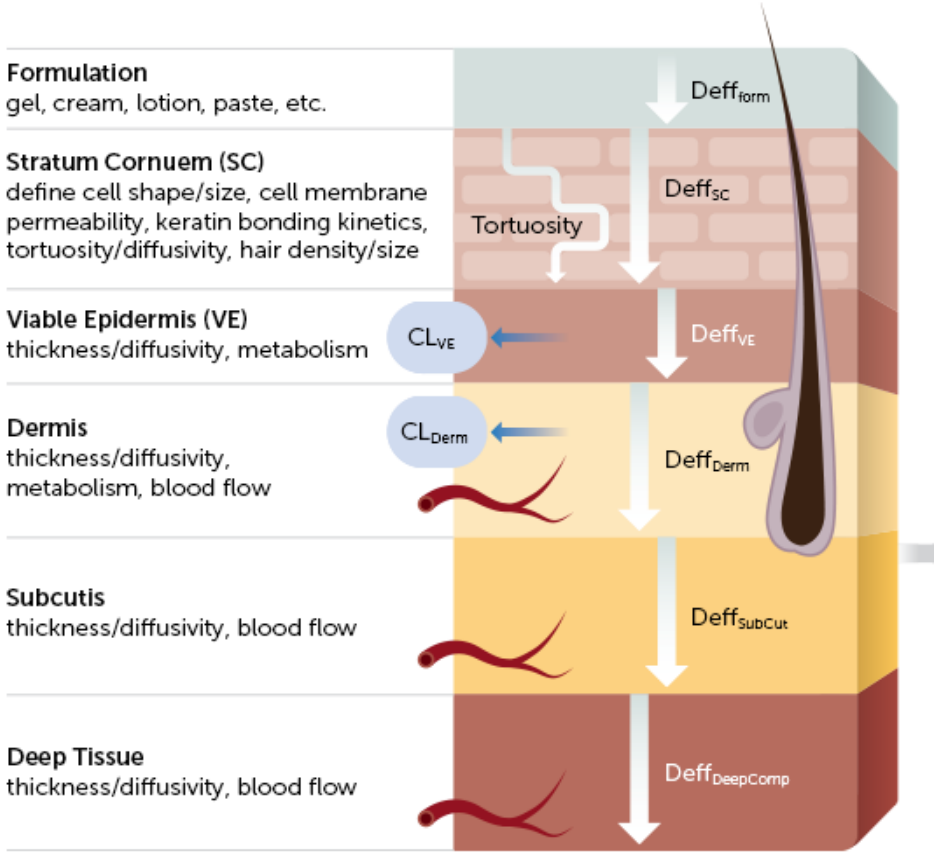
Background

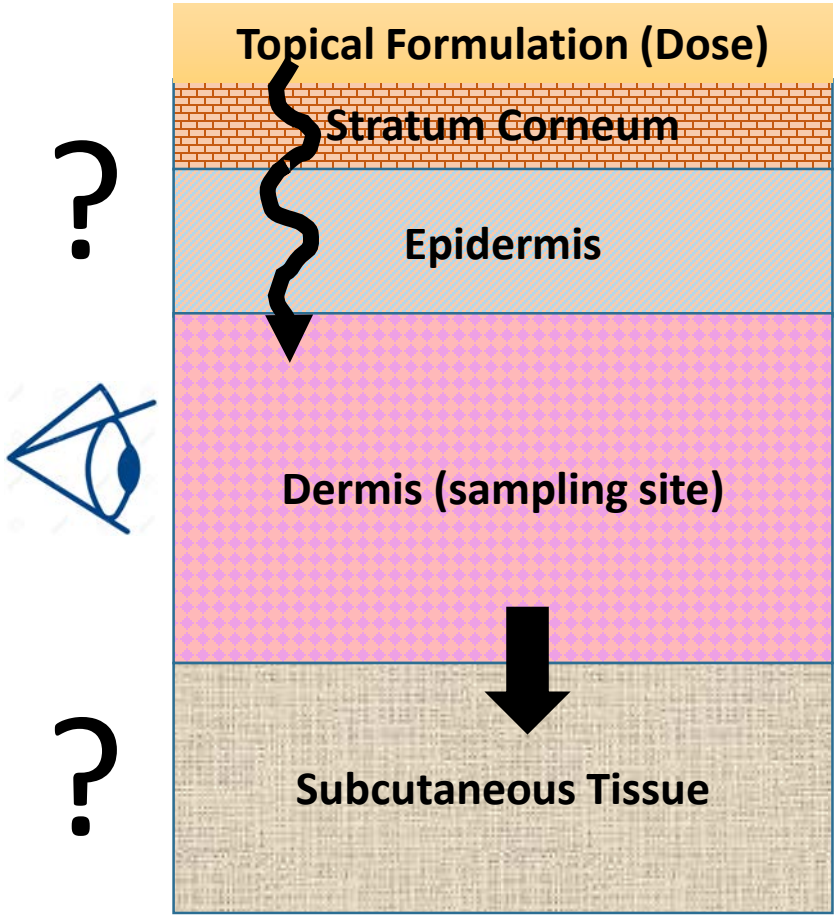
Rationale

- Skin pharmacokinetics following the topical application of dermatological products includes a number of processes



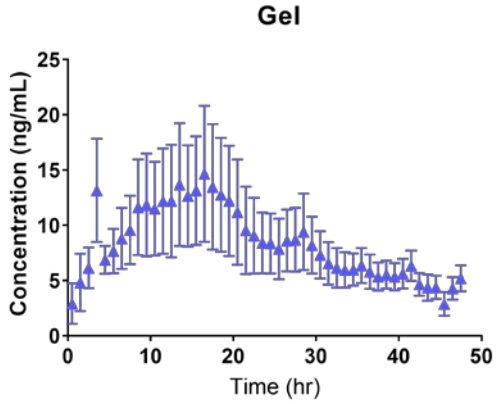
https://www.certara.com/2018/03/02/skin-in-the-game-mechanistic-modeling-of-dermal-drug-absorption/?ap%5B0%5D=PBPK

Experimental Data



Via microdialysis sampling, we observe the time course of the API in dermis, we have no data on what happens in the SC and epidermis, and how drug is eliminated

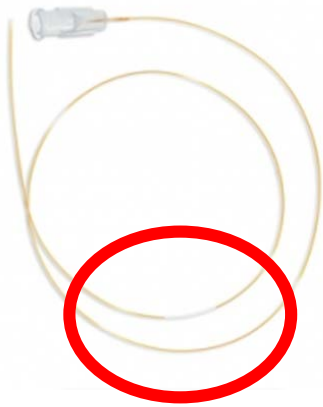
Observed concentration

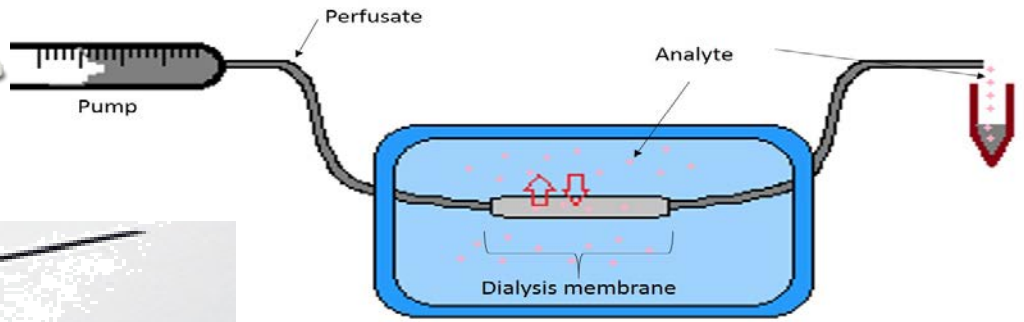


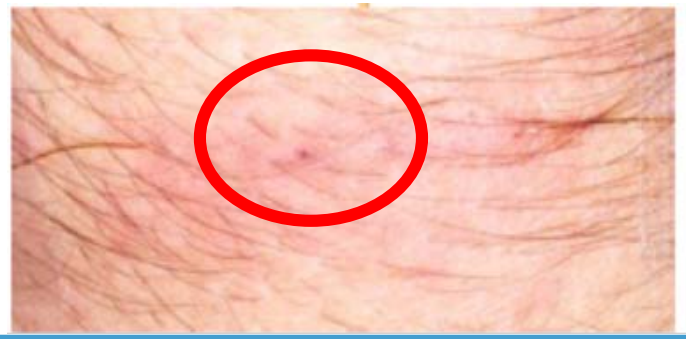
Empirically, we assume that the observed dermis concentration is the result of an absorption (input) function (f_t) and a disposition function (g_t): $C = (f * g)(t)$

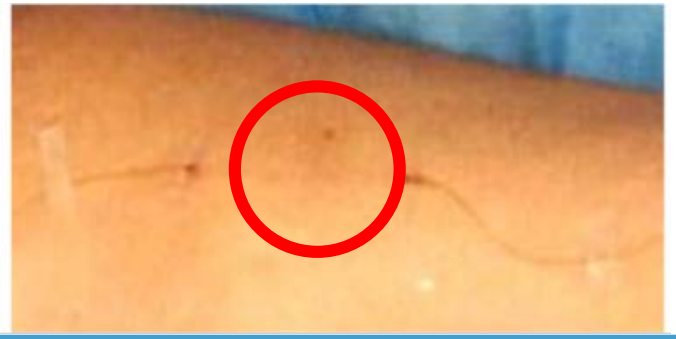
What is microdialysis?

- MD is an in-vivo, minimally invasive technique, that allows sampling of unbound molecules in dermis and subcutaneous tissues.

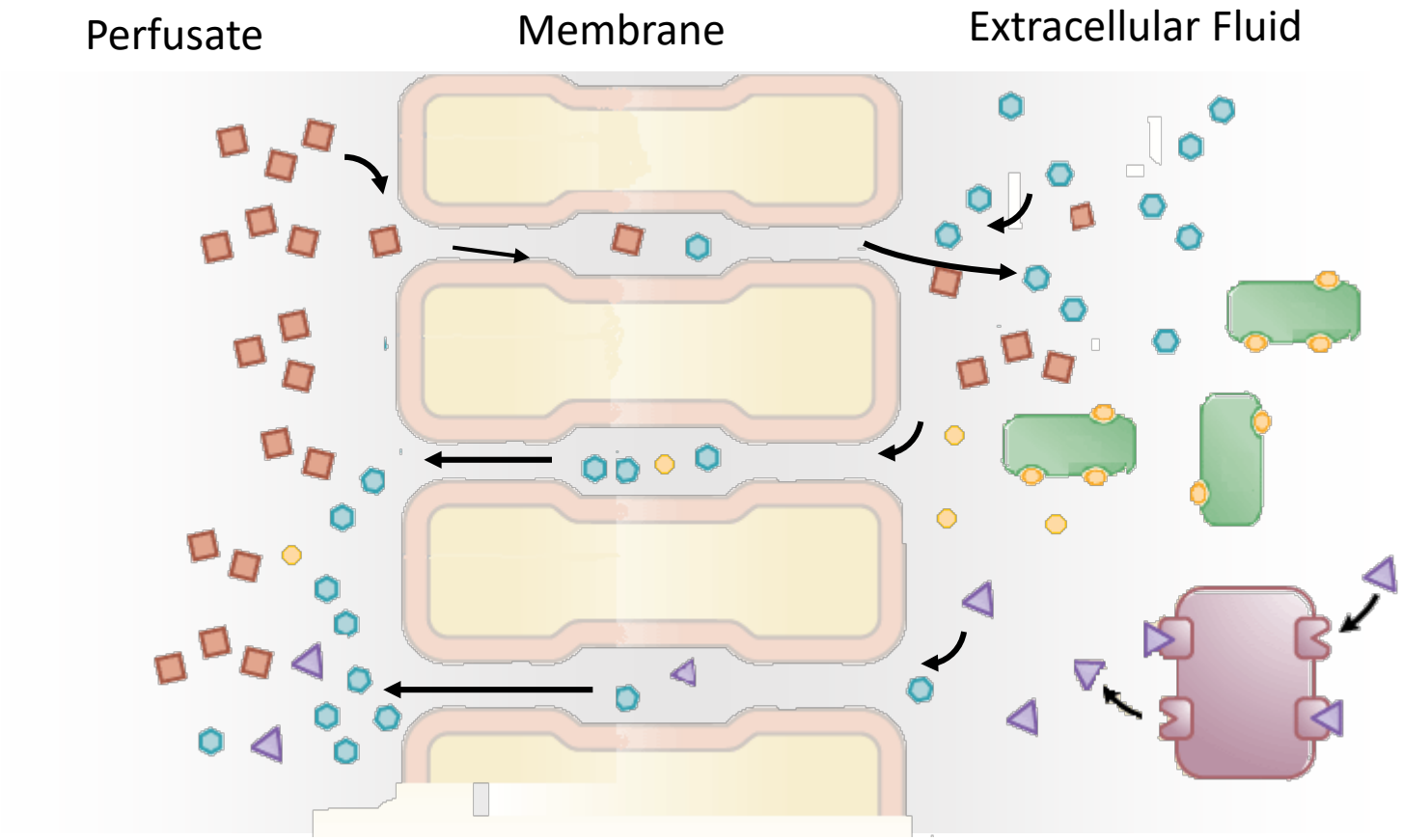




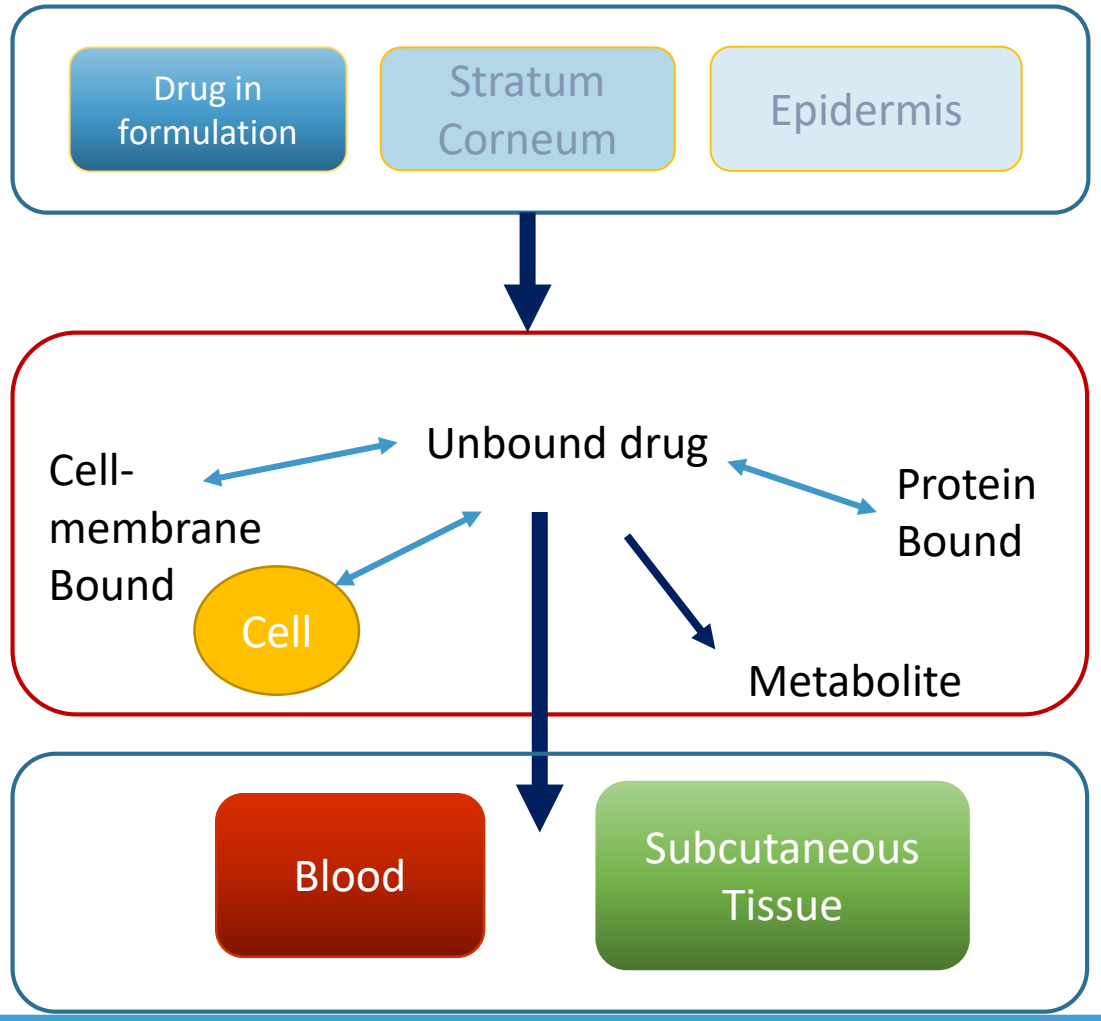




Microdialysis Principle



The observed dermis concentration profile results from:

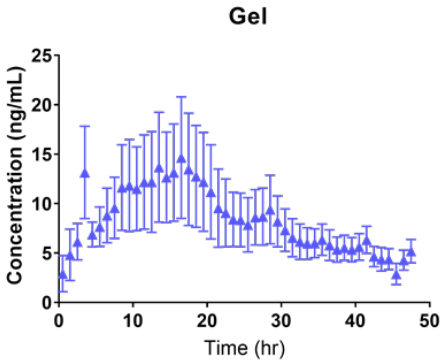


Absorption (f_t)

Distribution within dermis

Elimination

Disposition (g_t)



Definition and uses of Disposition Function

Disposition function (g_t) also know as "Unit Impulse Response" (UIR) is defined as:

- **The concentration deriving from the instantaneous administration of an unit amount of drug**: it accounts only for the distribution and elimination processes

In systemic (plasma) pharmacokinetics is estimated from iv-bolus or iv-infusions or simple oral formulations (simple 1st order absorption)

UIR is used in:

Convolution to predict the *in-vivo* plasma concentration profiles resulting from the administration of a selected input function (f_t):

$$C = (f * g)(t)$$

Deconvolution to estimate the *in-vivo* absorption profile or flux for topical delivery

$$f_t = (C/g)(t)$$

Typical use of UIR in the traditional IVIVC approach (FDA 1997)

Basic steps towards establishing IVIVC

- **Convolution**

  The convolution method is a simulation method used to predict the blood/plasma concentration using percent absorbed data

  solving $c(t)$ given $f(t)$ and $c_\delta(t)$

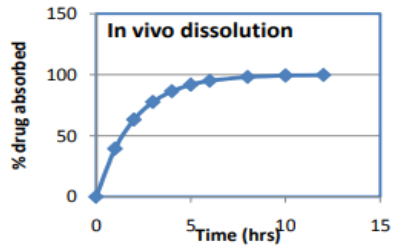
- **Deconvolution**

  Deconvolution is the process to obtain input function (percent absorbed) using known plasma concentrations

  solving $f(t)$ given $c(t)$and $c_\delta(t)$

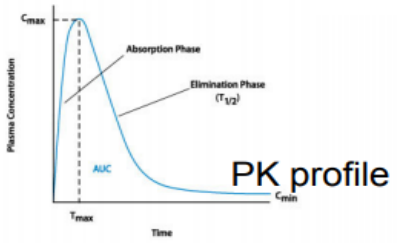
Deconvolution is the reverse process of convolution

$$c(t) = \int_0^t f(\tau) \cdot c_\delta(t-\tau) \cdot d\tau$$



Convolution

Deconvolution



Pfizer BIOPHARMACEUTICS

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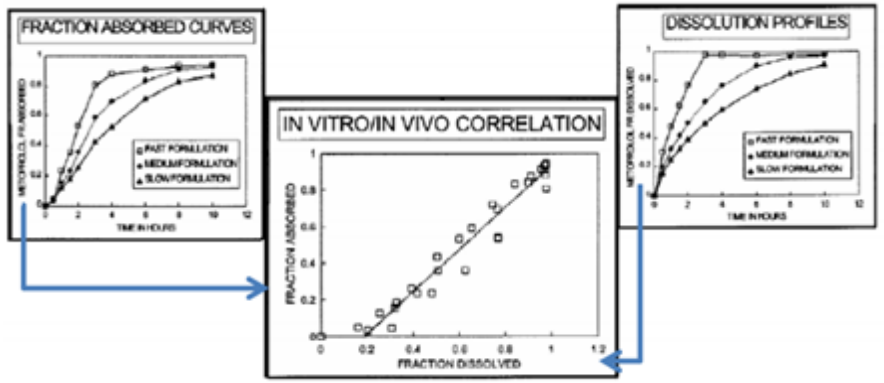
Ideal IVIVC



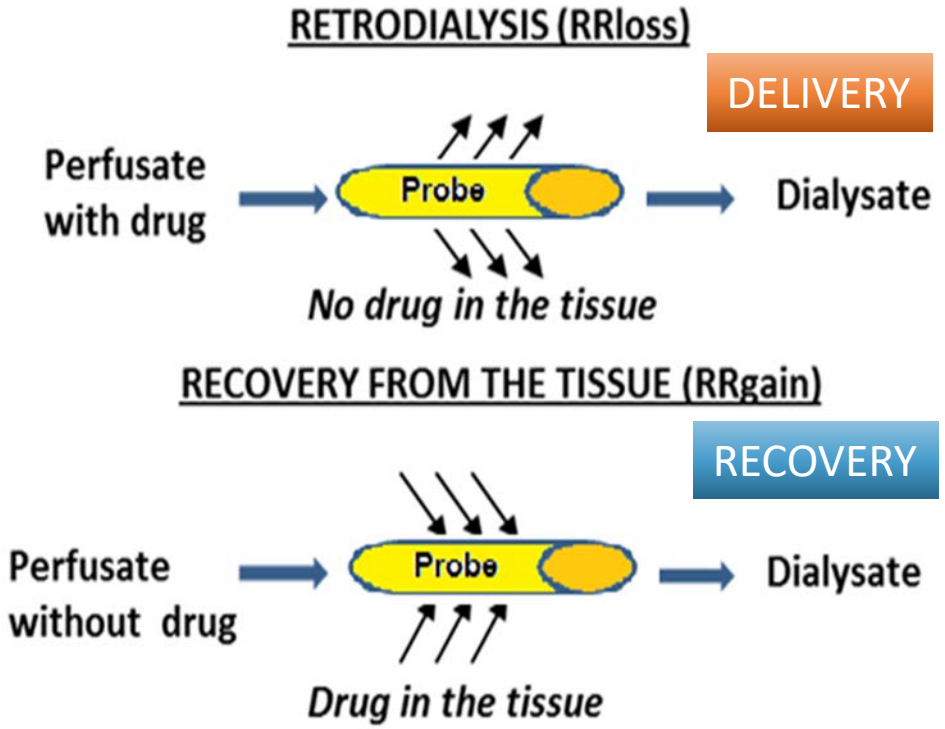
From: Rong Li, Pfizer Inc., Groton, CT (2015)

PBPK Models in IVIVC (oral)

- More recent IVIVC models use a physiological approach
- Absorption/PBPK modeling approaches differs from traditional convolution/deconvolution methods as they address in greater detail the absorption process
- **The part related to body disposition still needs estimates of volume of distribution and elimination in order to validate the model**
- Absorption/PBPK models do not overwrite empirical IVIVC approaches but rather complement them

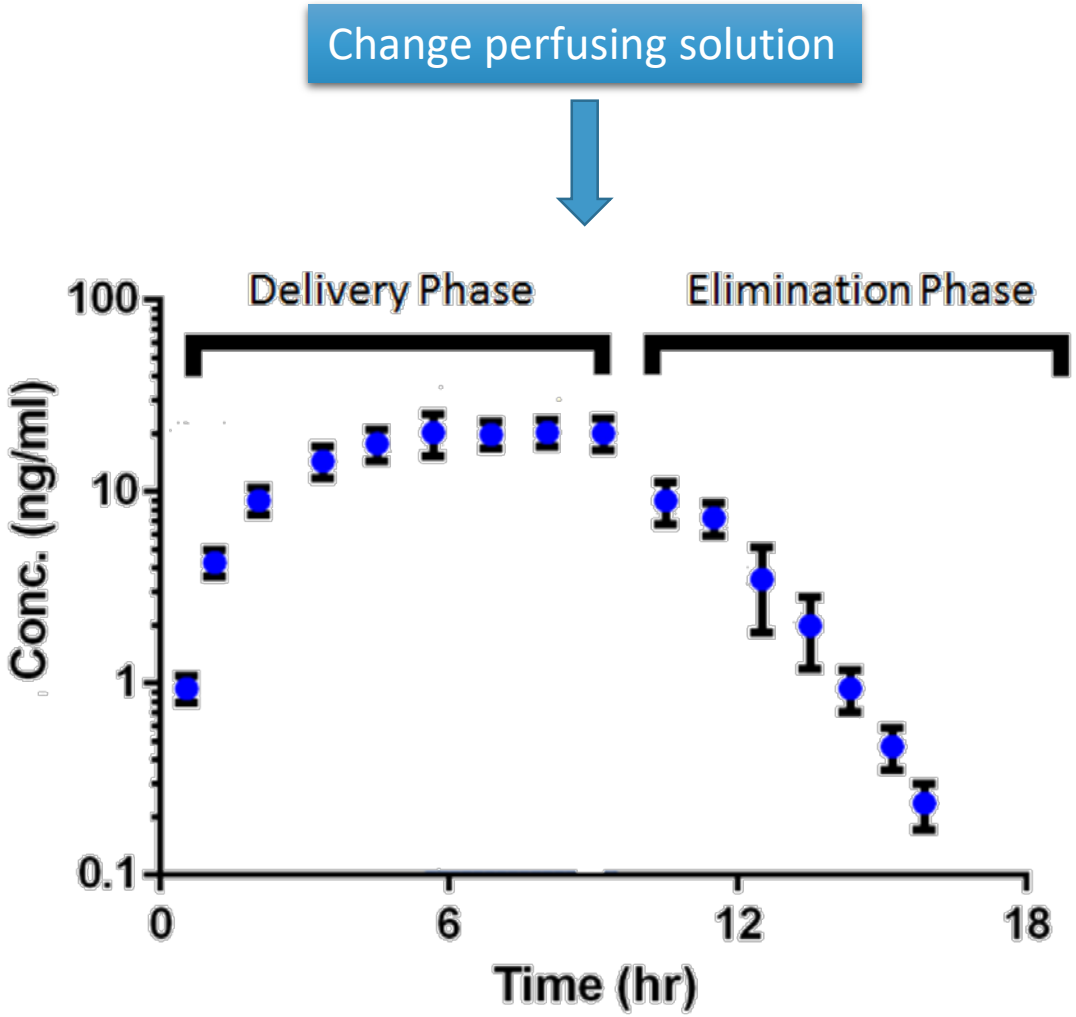
How can we estimate the disposition function (g_t) in dermis?

- In order to sort out the disposition function, it is necessary to administer the drug directly into the dermis, free from confounding factors due to the absorption process
- IDEA: use the microdialysis probe to deliver API directly in dermis



Study Design

- Insert a probe away from formulation application site
- Delivery Phase:
  - Add API to perfusate and deliver API via retrodialysis for enough time to have a well-defined steady state
- Elimination Phase:
  - Switch perfusing solution to plain Lactated Ringer solution
  - Continue sampling
- Analyze samples and plot data



Data Analysis

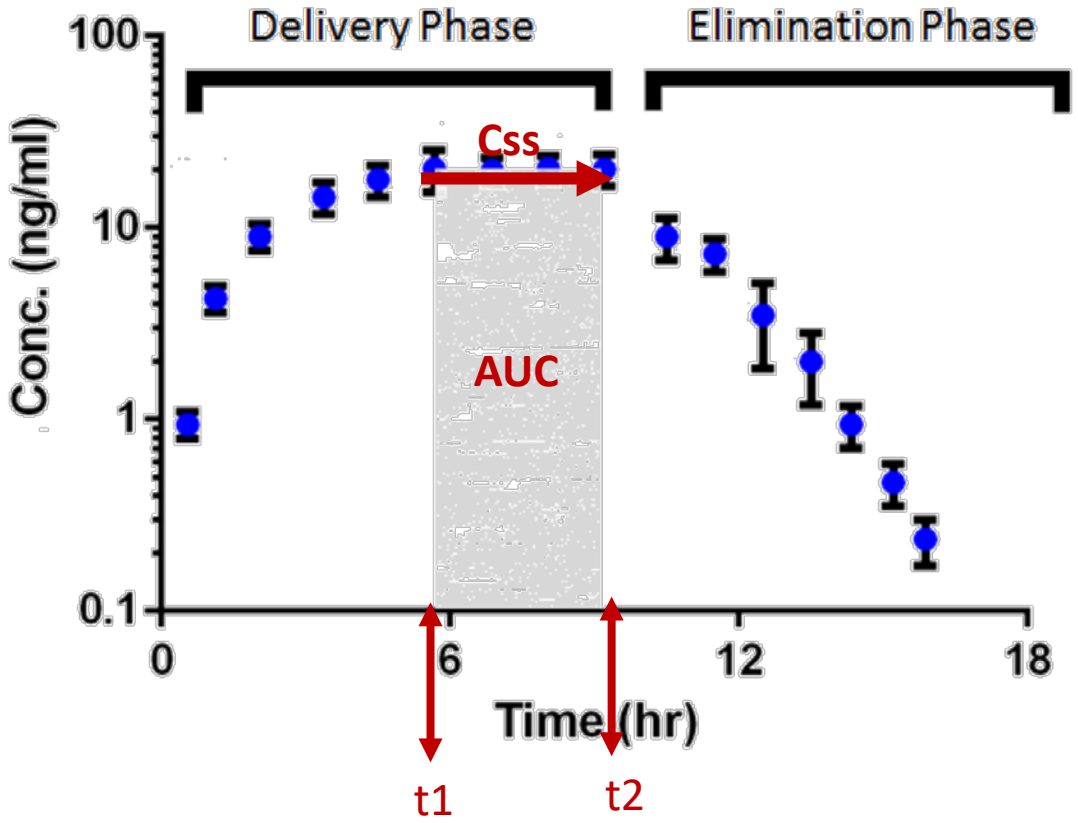
- Identify steady state concentration (C_{ss})
- Measure the AUC under the selected steady state (shaded area)
- Calculate the dose delivered in that time interval:

$$Dose_{t1-t2} = (C_{perfusate} - C_{ss}) \times V_{perfused\ (t1-t2)}$$

- Calculate clearance:

$$Cl = \frac{Dose_{t1-t2}}{AUC_{t1-t2}}$$

- Fit the best poly-exponential equation to the elimination-phase data;
- Estimate V_d:
  - E.g., if mono-exponential: $V_d = \frac{Cl}{k_e}$
  - More complicated if poly-exponential



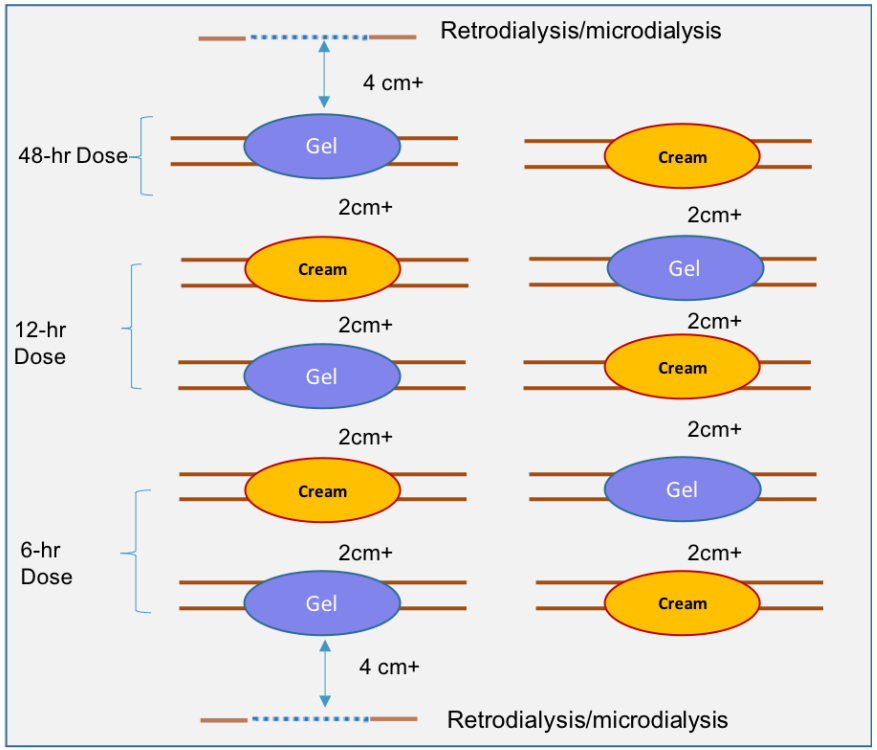
Purpose

- Estimate the dermis disposition function (dUIR) for metronidazole (MTZ) by utilizing microdialysis and retrodialysis techniques.
- Calculate the MTZ flux and cumulative amount permeated in vivo by deconvolution of the concentration profiles in the dermis.
- Correlate MTZ flux and cumulative amount permeated in vitro with the MTZ flux and cumulative amount permeated in vivo.

Methods

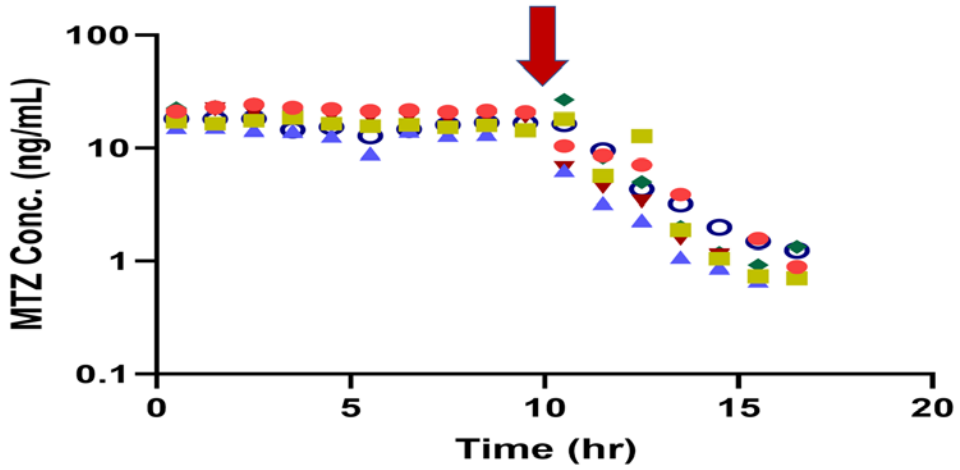
Experimental Design

- Three (3) Yucatan mini-pigs
- Twenty (20) probes placed under MTZ formulation (gel & cream) application sites. Three dosing-times were studied: 6hr when the formulation was wipe-off at t=6hr (6hr-dose), 12hr (12hr-dose), and 48 hr (no wipe off)
- Two (2) additional dMD probes inserted into the dorsum of each pig at a site distant from the formulations (previous studies indicated that no redistribution occurred)
  - These dMD probes were perfused with a 40 ng/mL solution of MTZ at a flow rate of 0.5µL/min for 10 hours (retrodialysis phase). The perfusion solution was then switched to plain lactated ringer solution and 20 ng/mL MTZ – D_3 solution as internal standard (microdialysis phase).
  - Samples were collected every hour until the end of the experiment (48 hr) and analyzed for MTZ content.



Results

Dermis Disposition Parameters



Dermal retrodialysis and microdialysis concentration vs time profiles. Data are per single probe.

- Dermis concentrations declined mono-exponentially following the delivery phase as the concentrations decrease in a straight line on a semi-log-scale.
- The average dose delivered between 5.5-9.5 hr was 3.5 ng $\pm$ 0.8 (n=6)
- The corresponding average $AUC_{5.5\text{-}9.5}$ was 62.81 ng*hr/mL (4.55) (geometric mean (CV%))
- The average volume of distribution was 0.12 $\pm$ 0.06 mL (mean $\pm$ SD)
- The average clearance from the dermis was 0.057 $\pm$ 0.03 mL/hr (mean $\pm$ SD)

Unit Impulse Response Calculation

- UIR for mono-exponential elimination:

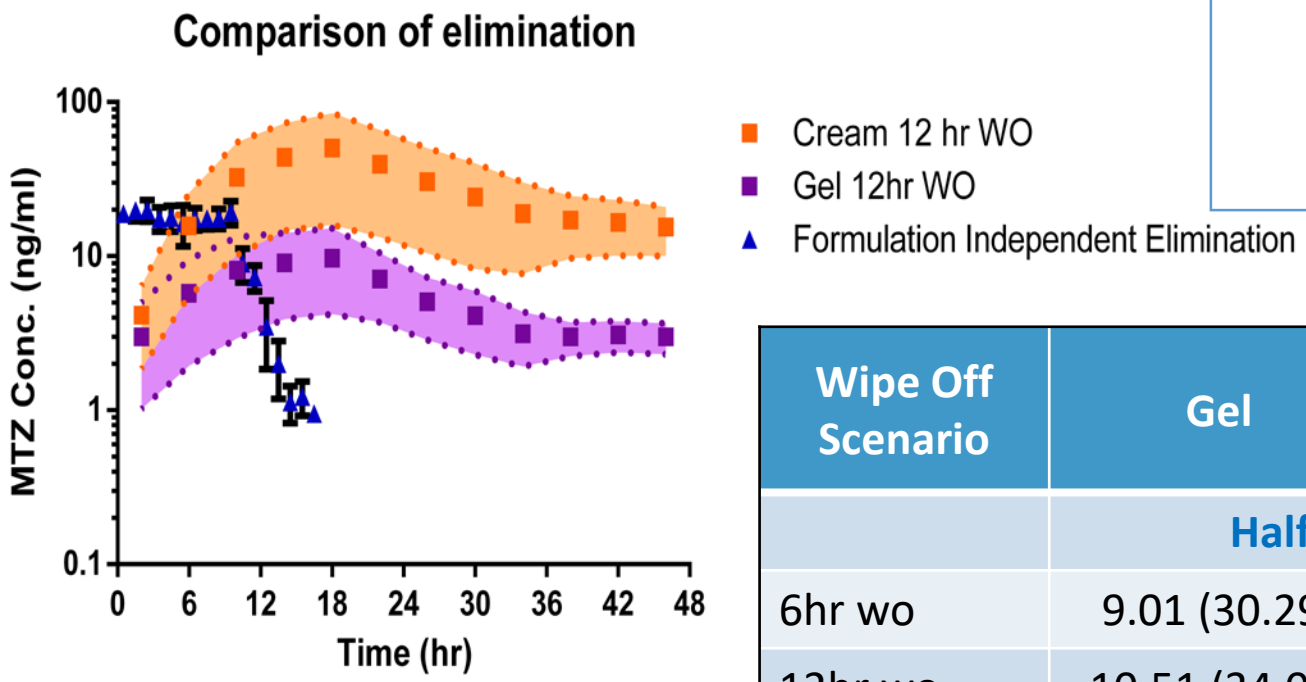
$$UIR = \frac{1}{V_d} \times e^{-k_e t}$$

Where V_d has units of mL and K_e has units of hr^{-1}

- Averaged UIR for all probes and subjects:

$$UIR = 10.1 \times e^{-0.45t}$$

Comparing Dermis Elimination Half-lives

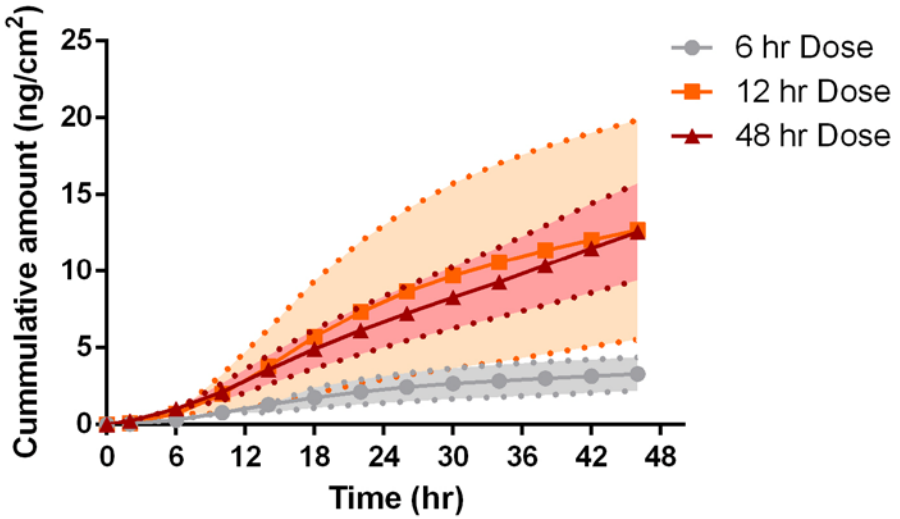


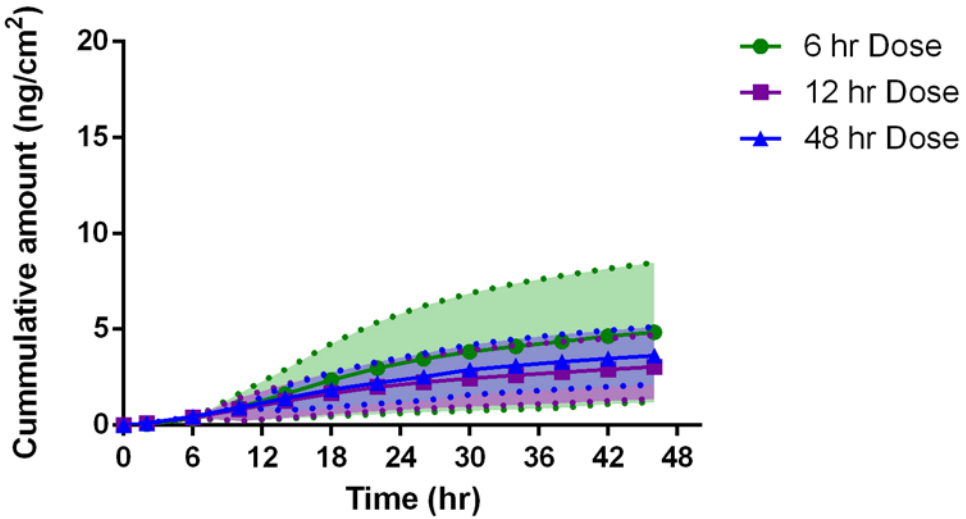
- Data indicate **Flip/Flop PK**:
  - Absorption (permeation) from upper layer of the skin is a prolonged, sustained process

Wipe Off Scenario	Gel	Cream	Formulation Independent
	Half-lives (hr) [geometric mean (CV%)]		
6hr wo	9.01 (30.29)	7.32 (25.95)	1.51 (23.1)
12hr wo	10.51 (34.06)	10.50 (57.75)	
No-wo	10.32 (32.84)	23.86 (86.74)	

Deconvolution[1] of Dermis Concentrations: **Cumulative Amount Input**

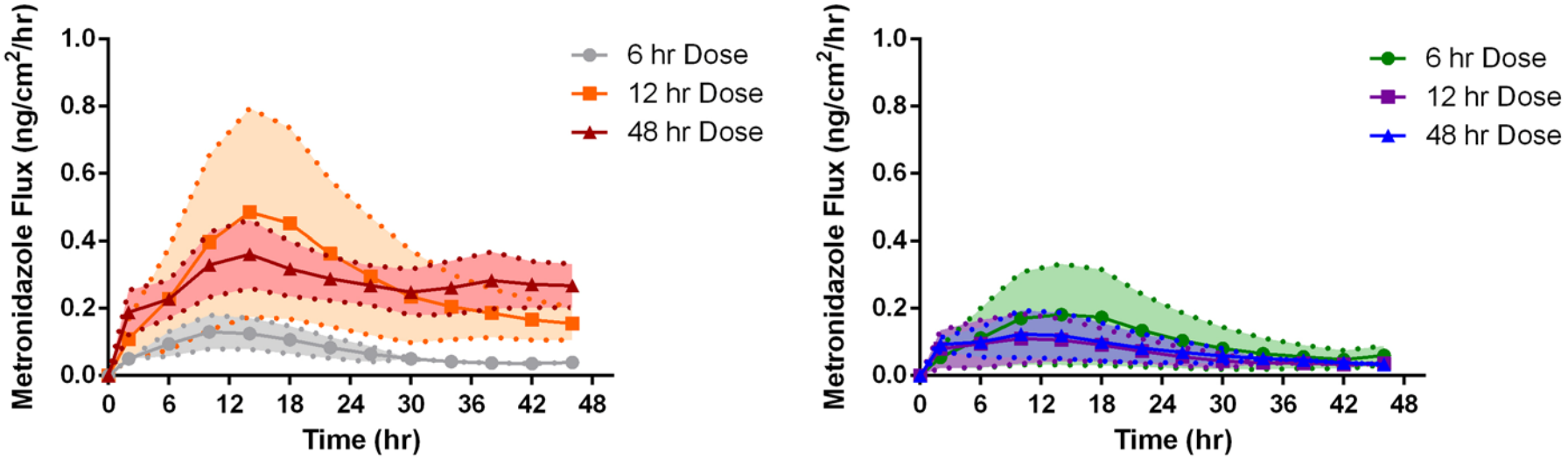
- **CREAM:** The 6hr dose delivered significantly less MTZ compared to the 12hr wipe off (p=0.038) and the 48 hr dose (p=0.046)
- **GEL:** There was no significant difference in the cumulative amount amongst the different dose times (p>0.667)





[1] Numerical Deconvolution performed with Phoenix®, Certara, Princeton, NJ

Deconvolution[1] of Dermis Concentrations: **In-vivo Flux**



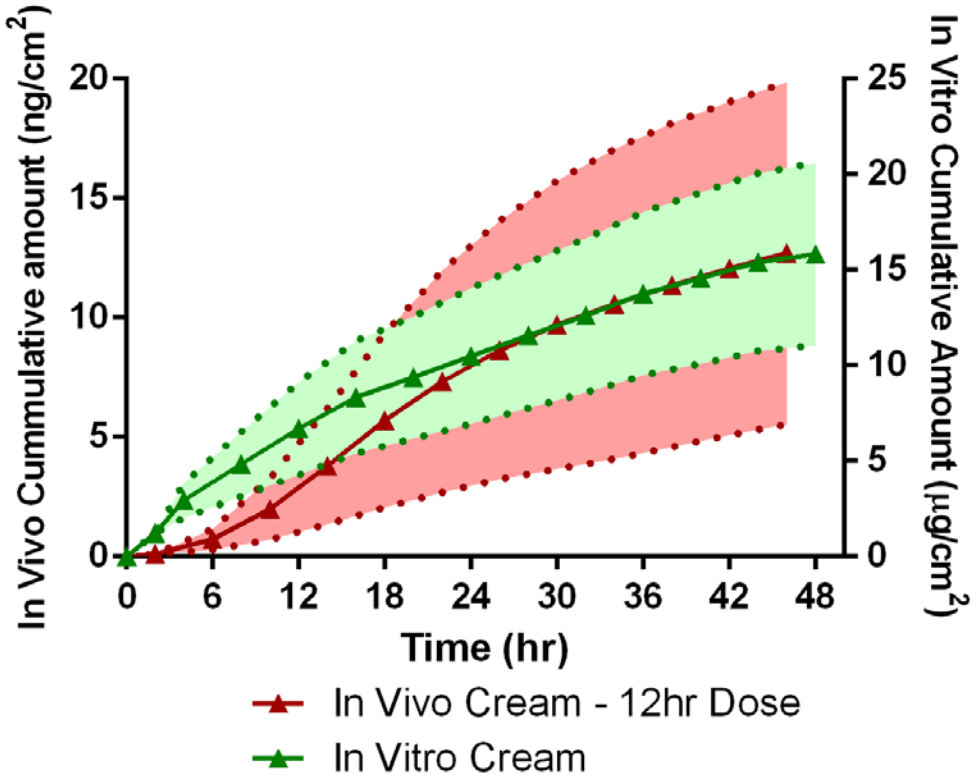
CREAM: The log transform of maximum flux [Ln(J_{max})] for the 6hr dose was significantly different from the 12hr dose (p=0.019) and 48 hr dose (p=0.041). The Ln(AUC) for the 6hr dose was also significantly different from the 12hr dose (p=0.018) and 48 hr dose (p=0.013).

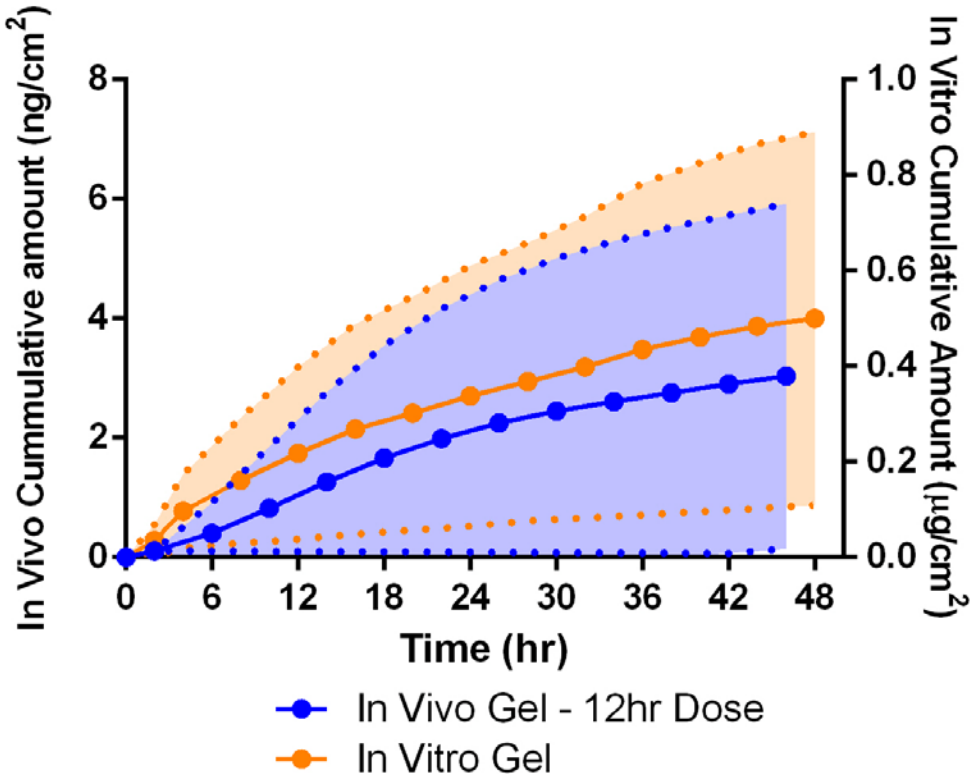
GEL: There was no significant difference amongst the different formulation wipe off schemes for Ln(J_{max}) (p>0.739) and Ln(AUC) (p>0.833)

CREAM/GEL: Comparison between the cream and the gel at the different dose schemes indicated that both Ln(J_{max}) and Ln(AUC) for the 48 hr dose were significantly different, *p*=0.010 and *p*=0.005, respectively; also at the 12hr dose scheme the Ln(J_{max}) and Ln(AUC) were significantly different between the formulations, p=0.02 and p=0.02, respectively; whereas at the 6hr dose scheme there was no difference between the two formulations.

[1] Numerical Deconvolution performed with Phoenix®, Certara, Princeton, NJ

Comparing in-vitro[2] and in-vivo cumulative amounts





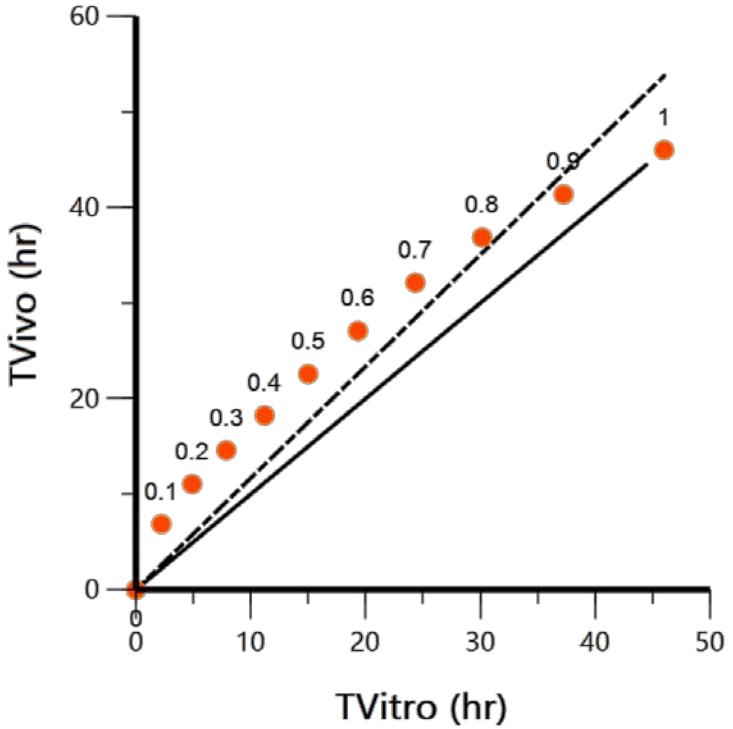
[2] In-vitro data courtesy of Dr. N. Murthy

Levy Plots

Levy plot of in vitro versus in vivo times needed to absorb the same fraction of API

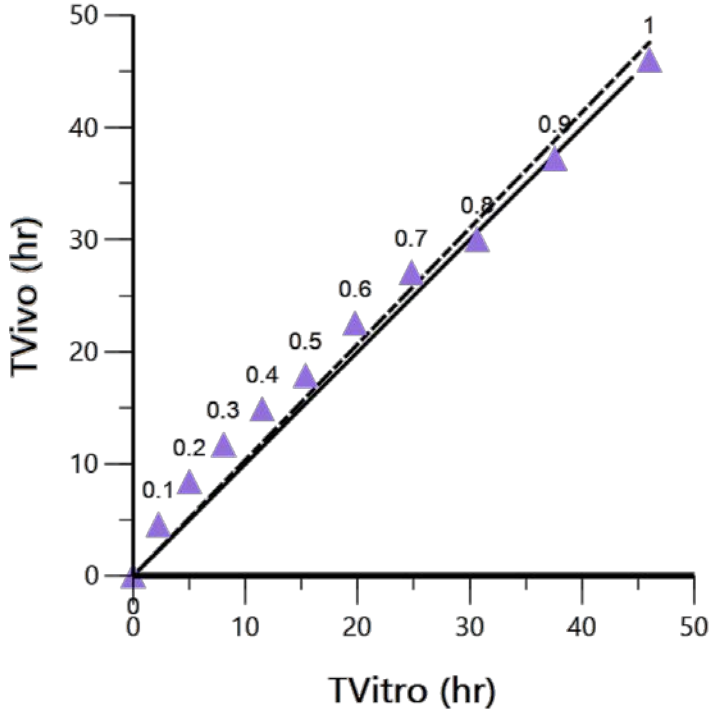
CREAM

Rsq = 0.972, Intercept = 0, Slope = 1.169



GEL

Rsq = 0.9917, Intercept = 0, Slope = 1.034

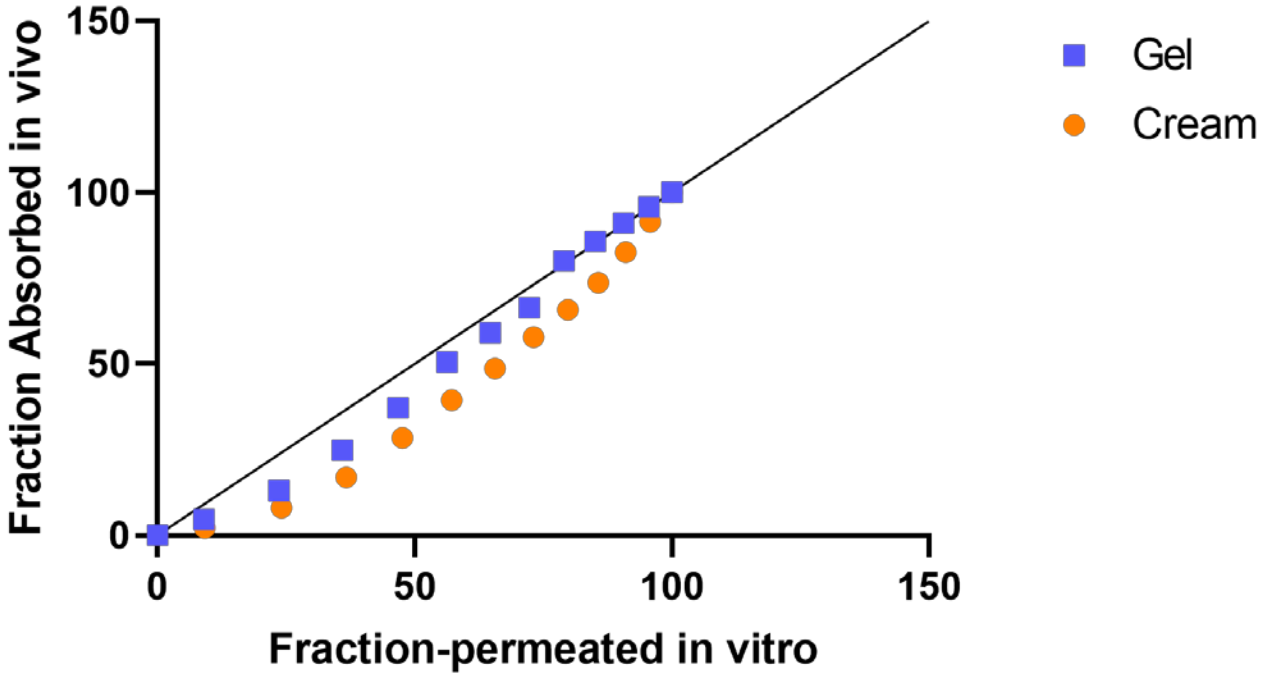


% Abs in-vivo vs % Permeated in-vitro

Unity Line

Regression

Levy Plots



- Levy plot of fraction permeated in vitro versus fraction absorbed in vivo for the cream and gel formulations
- The deviation of the data points from the unity line, indicates that a time scaling factor is necessary to build an IVIVR model

Conclusions

- The retrodialysis/microdialysis approach allows the estimation of formulation independent dermal elimination rate, volume of distribution, and clearance.
- The retrodialysis phase (delivery phase) provides a zero-order drug delivery directly to the dermis, from which the dose administered can be exactly calculated.
- The microdialysis phase allows the estimation of the actual elimination from dermis.
- Comparison of dermis elimination half-lives at the “formulation-independent” site with the half-lives measured at the topical formulation sites indicates a **flip-flop pharmacokinetic scenario** where the ”apparent” elimination phase reflects the absorption rate, which is the rate-limiting step in the dermis pharmacokinetics of these topical formulations of MTZ.

Developments

- The estimation of dermis disposition is a promising tool to estimate in-vivo flux and cumulative amount inputs that can be directly compared with in-vitro permeation data (IVPT) data.
- The characterization of the absorption process independent of local disposition may be helpful for the development of quantitative IVIVR using IVPT and dMD data and improve the comprehension of in-vivo percutaneous permeation that is crucial for the successful development of new formulations and for the assessment of bioequivalence of topical dermatological products.
- However, more refinement needs to be done in order to have an acceptable IVIVR to predict dermal drug flux from IVPT data

Acknowledgments

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