Impact of topical product metamorphosis on absorption of metronidazole

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BACKGROUND

- Topical products typically undergo significant transformation, or so-called "metamorphosis", after being "applied and rubbed" into the skin.
- Evaporation and permeation of solvents and excipients can lead to API supersaturation and/or precipitation. Metamorphosis can also impact the skin disposition of excipients (Figure 1).
- Drug delivery occurs from a "dynamic phase" evolving to the "residual phase", not from the product that was "in the tube"
- Understanding "metamorphosis" will:
  - Enhance understanding of drug delivery from the "residual phase"
  - Improve prediction of bioavailability and bioequivalence
  - Enhance simulation capabilities of current dermal PBPK models.

AIMS

- This work aimed to support development of dermal PBPK models by providing experimental data regarding the skin disposition and permeation of metronidazole (MTZ) when delivered from systems expected to metamorphose in different ways (Fig.1).
- For this:
  - "Fast-metamorphosis" ethanol : water-based vehicles, and "slow-metamorphosis" propylene glycol : water-based vehicles were formulated as either liquid or gelled systems.
  - The dynamics of MTZ uptake into the SC and viable tissue, and delivery of the drug to the receptor compartment were characterized.

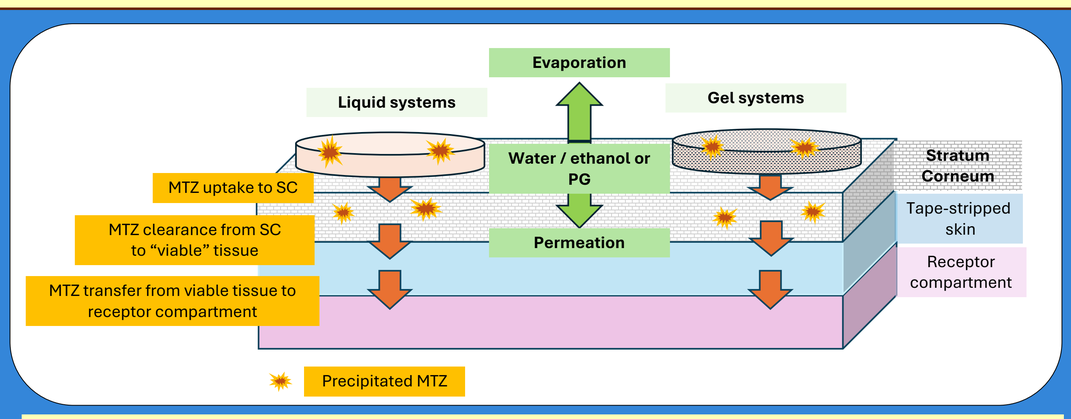

Fig.1: Skin permeation and metamorphosis events anticipated to contribute to MTZ skin disposition and permeation from the systems used in this work.

METHODS

Solubility

- MTZ solubility in different vehicles was determined by adding excess drug to pure solvents, binary mixtures and gels.

In Vitro Permeation Tests (IVPT)

- Experiments (n = 3 – 4 replicates) investigated the skin disposition of MTZ when delivered from vehicles (Table 1) containing the drug at the same initial thermodynamic activity (90% of its saturation solubility) for the following conditions:
  - Dermatomed abdominal porcine skin,
  - Vertical, Amber glass, Franz diffusion cells, 1.77 cm^2 transport area,
  - Donor: 110 μL and 120 μL for liquid and gel vehicles, respectively,
  - Receptor: pH 7.4 – PBS buffer,
  - 32°C and 44% relative humidity,
  - Sampling: MTZ in (a) receptor solution throughout the experiment, and (b) SC (20 tape-strips) and tape-stripped ("viable") skin at the end of the experiments.
- Similar experiments (without MTZ) were performed to assess PG presence in the SC and viable tissue.
- HPLC was used to quantify MTZ and PG (1, 2).

Table 1: Vehicles selected for permeation studies; drug load and duration of uptake for each vehicle.

Vehicle	Ethanol:water 25:75 (v:v)	Ethanol:water 25:75 (v:v) + 4%HEC	PG:water 50:50 (v:v)	PG:water 50:50 (v:v)	PG:water 50:50 (v:v) + 4%HEC	PG (100%)	Water (100%)
MTZ (mg/mL)	18.44	15.79	21.07	0	15.68	24.65	10.55
Uptake duration (h)	1, 2 and 4	4	2, 4 and 24	4 and 24	4 and 24	4	4

Confocal Raman microscopy mapping experiments.

- A series of preliminary experiments explored the potential use of this technique to assess the physical state of MTZ in the residual phase.
- Skin was treated with the PG:water gelled formulation for 4 hours and then imaged. Data were acquired using confocal Raman spectroscopy (Renishaw RM1000 Raman microscope running v4.4 WIRE software, Renishaw plc, Wotton-Under-Edge, UK) in a 'top-down' approach (3).
- Signals for solid (1185 cm^{-1}) and dissolved 1192 cm^{-1} (C-N stretching) MTZ, PG (840 cm^{-1}), phenylalanine (1004 cm^{-1}) and HEC (1450 cm-1) were tracked across the skin surface.

RESULTS

Solubility data

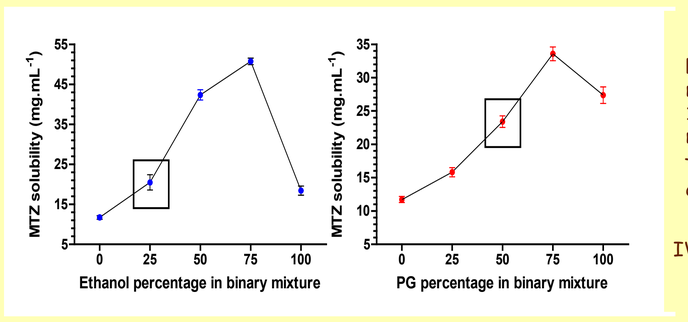

Fig.2: MTZ solubility in water, ethanol, PG and binary mixtures. Left panel: Ethanol : water (mean ± SD of n = 3-13). Right panel: Propylene glycol (PG):water binary mixtures (mean ± SD of n = 6-9).

The box indicates the binary mixtures selected for IVPT experiments and for preparation of the gel formulations.

IVPT formulations were selected based on these results.

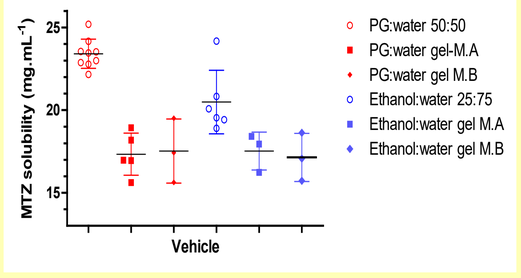

Fig.3: MTZ solubility at 32°C (each replicate and mean ± SD) in the vehicles selected for IVPT experiments. PG:water 50:50 (n = 9); PG:water gel (Method A, n = 5); PG:water gel (Method B, n = 3); ethanol:water 25:75 (n = 6); ethanol:water gel (Method A, n = 5); ethanol:water gel (Method B, n = 3).

Methods A and B differed in the time (0 vs 24 hours) at which the gelling agent was added to the mixture. No differences were found between MTZ solubility in gels prepared by the two methods.

IVPT data: Permeation Data

PG:water vehicles — Gelled vs non-gelled systems

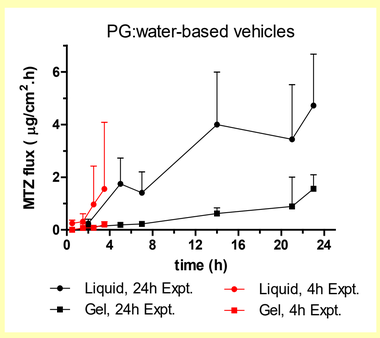

Fig.4: MTZ flux into the receptor as a function of time (midpoint of sampling interval) for the PG/Water (50:50 v/v) binary solvent mixture and corresponding gel (mean ± SD, n = 3-4).

Uptake duration \ Vehicle	PG:water 50:50 (v:v)	PG:water 50:50 (v:v) + 4%HEC
4 h	3.1 ± 4.2	0.31 ± 0.17
24 h	72 ± 32	14 ± 2.2

Table 2: Cumulative MTZ delivery (μg/cm^2) from the PG/Water (50:50) solvent mixture and corresponding gel (mean ± SD, n = 3-4 replicates).

MTZ cumulative delivery decreased for the gel vehicle at both 4 and 24 h ($p < 0.004$, Two-Way ANOVA) (Table 2, Fig.4)

PG uptake

Uptake duration	SC (μg/cm^2) [tapes 1-2 excluded]	Stripped skin (μg/cm^2)
4 h	138 ± 29 [92 ± 14]	11.9 ± 10.8
24 h	48 ±32 [35 ±25]	10.8 ± 0.12

Table 3: PG skin uptake in the 4 and 24 h IVPT experiment conducted with the PG:Water (50:50 v/v) vehicle in the absence of MTZ. (mean ± SD, n = 3 replicates)

All vehicles compared — 4 h Uptake

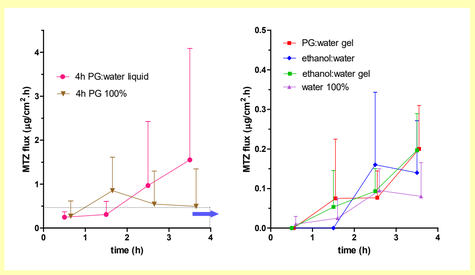

Fig.5: MTZ fluxes into the receptor as a function of time (midpoint of sampling interval) for all the vehicles tested (mean ± SD, n = 3-4 replicates).

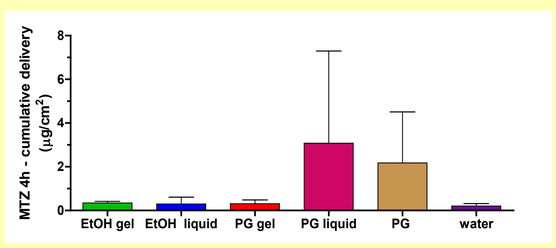

Fig.6: MTZ cumulative delivery to the receptor for all the vehicles tested. No significant differences were found among these data (mean ± SD, n = 3-4 replicates).

IVPT data: SC uptake and "viable" tissue recovery

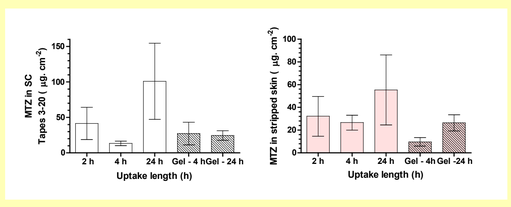

Fig.7: MTZ recovery from SC (excluding tapes 1-2) and from skin post-stripping at the end of IVPT experiments conducted with PG:Water (50:50 v/v) binary solvent mixture and the corresponding gel. Data are mean ± SD (n = 3-4 replicates).

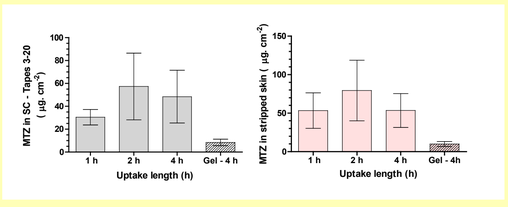

Fig.8: MTZ recovery from SC (excluding tapes 1-2) and from skin post-stripping at the end of IVPT experiments conducted with Ethanol:Water (25:75 v/v) binary solvent mixture and the corresponding gel. Data are mean ± SD (n = 3 replicates).

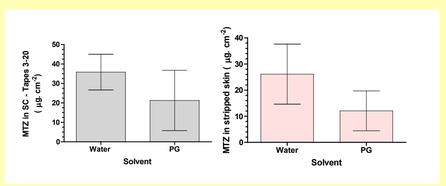

Fig.9: MTZ recovery from SC (excluding tapes 1-2) and from skin post-stripping following 4h IVPT experiments with pure water and PG vehicles. Data are mean ± SD (n = 3-4)

Preliminary Raman mapping data

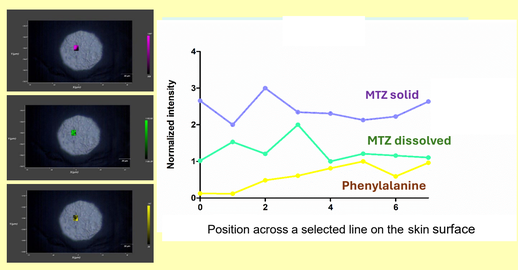

Fig.10: Raman mapping: The normalized intensities of the deconvoluted signals at 1185 cm^{-1}, 1192 cm^{-1}, and 1003 cm^{-1} attributed to solid MTZ, dissolved MTZ and phenylalanine along a line within the selected skin surface area are shown.

The skin was treated with the PG:Water (50:50 v/v) gel for 4 hours before imaging.

The signal for solid MTZ was registered in areas where no drug crystals were apparent. This suggests that the signal corresponds to micro- or nano-particles.

Further method development is required to characterize MTZ status in the residual phase as metamorphosis proceeds.

CONCLUSIONS

(1) MTZ skin disposition and permeation differed across vehicles in which MTZ was incorporated at the same initial thermodynamic activity. Suggested explanations for these differences are the dissimilar metamorphosis (evaporation of solvents and impact of gelling agent) experienced by these vehicles that lead to different patterns of MTZ precipitation in the residual phase as it is formed. In addition, the dynamics of PG skin disposition is another, potential contributing factor.

(2) Further studies aim to elucidate the dynamic changes in vehicle composition and in MTZ physical state as metamorphosis proceeds.

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