Topical product metamorphosis: impact on metronidazole *in vitro* skin permeation post-application of binary mixture formulations

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Background and Purpose

Metamorphosis of topical drug products after their application to the skin involves a physical and compositional transformation to a residual film, impacting the resulting cutaneous pharmacokinetics and local bioavailability of the active pharmaceutical ingredient. Metamorphosis occurs in both simple vehicles and conventional formulations, such as creams and gels [1]. Evaporation/skin permeation of inactive ingredients and changes in the drug's solubility are elements of this metamorphosis that require careful characterization. The long-term aim of this research is the acquisition of experimental data associated with topical product transformation that can inform mechanistic *in silico* models of drug uptake into the skin, permeation and absorption into the systemic circulation. Herein, we present a case study using simple ethanol/water (EtOH/H_2O) and propylene glycol/water (PG/H_2O)-based formulations of metronidazole (MTZ), an active commonly used to treat rosacea and other skin diseases.

Materials and Methods

The solubility of MTZ in the selected vehicles/mixtures was determined as previously reported [2]. Briefly, excess quantities of MTZ were added to the relevant solvent ($n \geq 3$) and stirred for 24 h at 32°C in sealed vials protected from light. Samples were then filtered and the dissolved MTZ concentrations were determined using HPLC.

1-, 2- or 4-hours *in vitro* permeation testing (IVPT) studies used dermatomed abdominal porcine skin, formulations containing MTZ at 90% saturation (applied dose 62.15 µL/cm^2), and Franz diffusion cells. MTZ permeation into the receptor solution (pH 7.4 phosphate-buffered saline), and MTZ uptake into the viable skin and into the stratum corneum (SC, tape-stripping - 20 tapes), were determined.

Results and Discussion

MTZ solubilities in EtOH/H_2O mixtures (Figure 1), 25/75, 50/50, and 75/25 (v/v), were significantly higher than in the pure solvents ($p<0.05$), demonstrating strong co-solvency effects in these mixtures. The results align with previous reports that MTZ solubility reached a maximum at 80% v/v EtOH/H_2O that was greater than the value in pure EtOH [3].

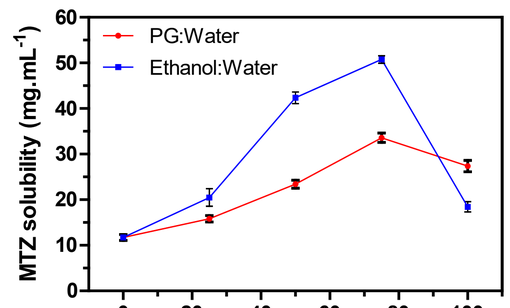

Figure 1. Solubility (mean ± SD) of MTZ in EtOH/H_2O (n=3-13) and PG/H_2O (n=6-9) formulation binary solvent mixtures.
All solubility values within each series are significantly ($p<0.05$) different to each other.
Data are shown as mean ± SD

MTZ solubility increased with increasing the PG content in PG/H_2O mixtures. However, in contrast to EtOH/H_2O mixtures, MTZ solubilities in PG/H_2O 25/75 and 50/50 mixtures were lower ($p<0.05$) than that in pure PG.

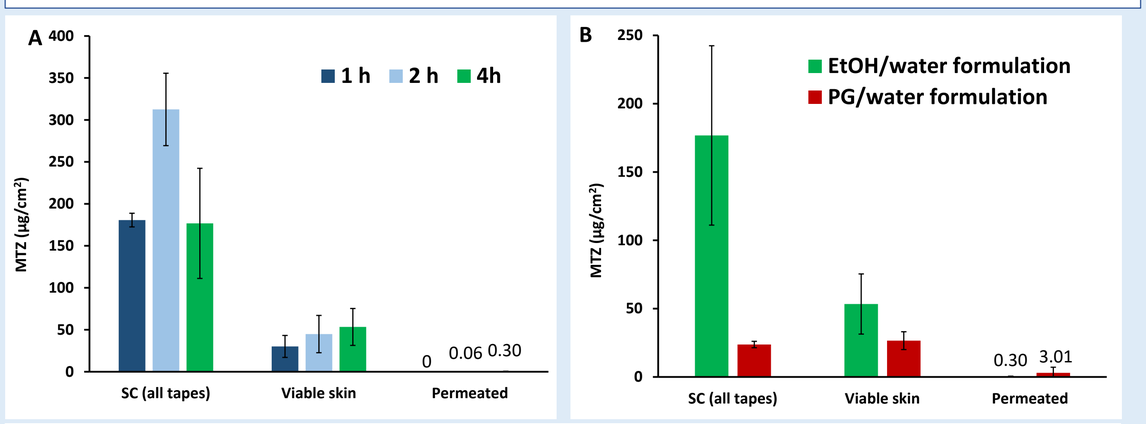

Figure 2. IVPT results comparing (A) EtOH/H_2O formulations (25/75 v/v) after 1h, 2h, and 4h application times, and (B) EtOH/H_2O (25/75 v/v) and PG/H_2O (50/50) after 4 h of application. Data shown as mean ± SD (n=3 replicates).

When the 25/75 EtOH/H_2O mixture initially containing MTZ at 90% of its saturation solubility was applied to the skin, precipitation of the drug on the skin surface was visible within the first hour as the solvents evaporated from the skin surface and/or penetrated into the SC. Also, MTZ recovered from the SC was larger ($p<0.05$) at 2 h than at 1 and 4 hours (Fig.1A). MTZ recovered from the SC at 4 hours was larger for the EtOH/H_2O than for the PG/H_2O donor (Fig 1.B), No other differences were found.

MTZ delivery across the skin from EtOH/H_2O (Figure 2A) increased with application time and the drug was detectable in the receptor solution after 4 hours.

Application of MTZ for 4 hours at 90% saturation in PG/H_2O (50/50 v/v) resulted in lower ($p<0.05$) SC uptake of the drug but greater delivery into the receptor than that achieved with the EtOH/H_2O (25/75 v/v) vehicle (Figure 2B).

The differences between the two vehicles, in which the drug was initially at the same thermodynamic activity, can be related to differences in metamorphosis events – including MTZ precipitation – occurring on the skin surface. It is possible that at least some of the MTZ recovered from SC sampling post-application of the EtOH/H_2O formulation represents drug precipitated in skin 'crevices' to which this less viscous vehicle would have easier access.

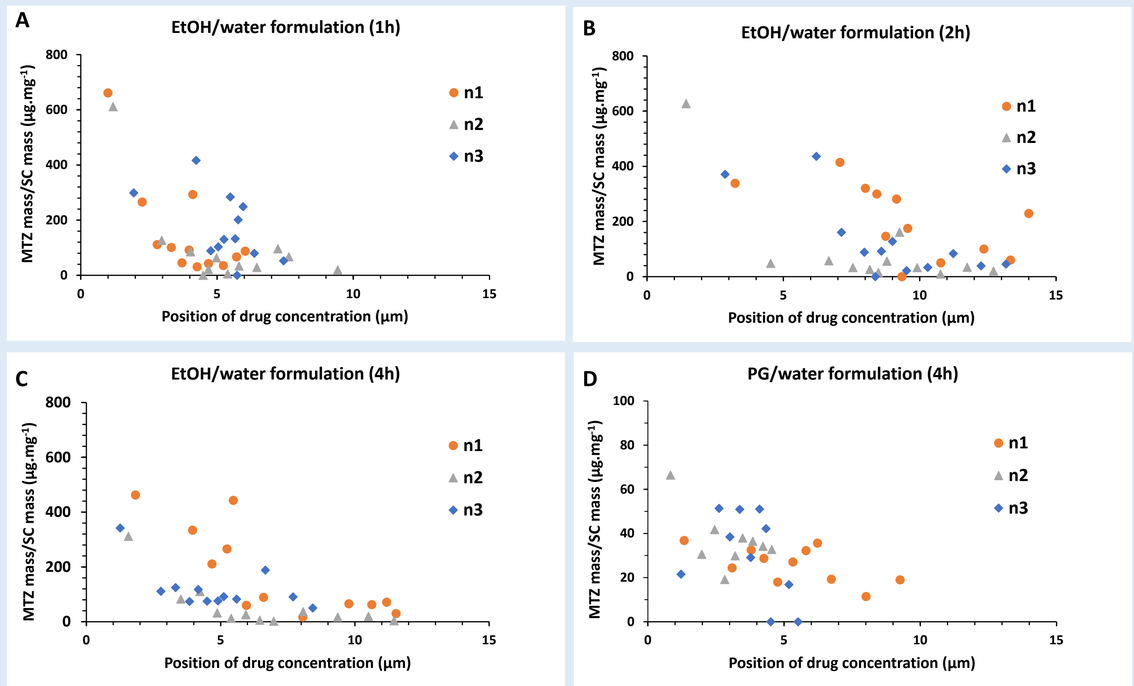

Figure 3. MTZ concentration (µg.mg^{-1}) as a function of SC depth for EtOH/water 25/75 (v/v) after (A) 1 h, (B) 2 h, and (C) 4 h application times, and (D) for PG/H_2O 50/50 (v/v) formulation after 4 h application. n1, n2, and n3 indicate three replicate experiments for which each replicate is plotted, for three experiments presented. Note the different Y-axes for panels A-C and D.

MTZ concentration versus SC depth profiles for the EtOH/H_2O (25/75 v/v) formulation showed deeper permeation of the drug at 2- and 4-hour (Figures 3B and 3C) compared to 1 hour application (Figure 3A), consistent with the receptor data. MTZ concentration versus depth profiles were lower for the PG/H_2O (50/50 v/v) formulation (compared with the EtOH/H_2O) for the same application times (Figure 3C vs 3D).

Conclusion and Future Work

Preliminary data suggest that MTZ uptake and distribution in the SC and underlying skin layers were different for the two vehicles (EtOH:water and PG:water) despite the same initial thermodynamic activity of the active pharmaceutical ingredient. Contributory factors may be different solvent evaporation rates, MTZ precipitation post-application of the EtOH-based vehicle, and differential access of the formulations to skin 'crevices'. Further experimental work aims to understand the metamorphosis events occurring during MTZ skin permeation from different formulations.

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