Solvent Enhancement Effect From Finite Dose Applications

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Introduction and Purpose

The use of penetration enhancers is a well-known strategy to improve delivery across human skin. In spite of extensive literature on permeation enhancers and their mechanisms of action, little has been published on the effect of the enhancer when applied at clinically relevant doses (typically less than a few mg/cm^2). A recent study (Trottet et al., 2004) has shown that the depletion of propylene glycol from the formulation can limit its enhancing effect, especially when relatively small doses are used. The authors also suggested that the penetration enhancement of solvents may be overestimated in *in vitro* studies because most studies are performed under infinite dose conditions.

The purpose of the present investigation was to study the enhancement effect of Transcutol P® (TC), dimethyl isosorbide (DMI), isopropyl myristate (IPM) and ethanol (Et) on the permeation of methyl paraben (MP) across silicone membranes, when applied at clinically relevant doses (finite doses).

Materials and Methods

Methyl paraben (Methyl-4-hydroxybenzoate puriss. ≥99%, Fluka) and IPM (Isopropyl Myristate 98%, Aldrich) were supplied by Sigma-Aldrich, UK. Dimethyl isosorbide (Arlasolve® DMI) and Transcutol P® were supplied by Uniqema and Gattefossé, respectively. Ethanol (99.7 - 100% v/v AnalaR® grade, BDH) was supplied by VWR UK. PBS was prepared *in situ* by dissolving 10 Phosphate Buffered Saline (Dulbecco A) tablets (pH 7.3±0.2 at 25oC, Oxoid) supplied by Fisher Scientific UK in 1 litre of deionised water (diH_2O). The silicone membranes (aprox. 250 µm thickness) were supplied by Samco, UK.

Saturated solutions were produced by adding excess amount of solute to each solvent with stirring for at least 24 hours at 32 (±0.5)°C, after which the suspended drug crystals were removed by filtration. The permeation experiments were conducted at 32 (±1)°C using Franz-type diffusion cells (0.801 cm^2 diffusion area). The silicone membranes were pre-soaked overnight in PBS. Finite dose studies were conducted by applying small volumes of the donor solutions (12.5 µl/cm^2) which were evenly spread at the membrane surface using a micropipette (finite dose study). Infinite dose studies were performed using 1ml of a saturated suspension of methyl paraben in water (containing undissolved drug to ensure maintenance of saturation during the experiment). Permeation from neat powder was studied by applying 4.6 mg of methyl paraben per cm^2. Sampling of the receptor occurred at designated time points with volume replacement using fresh PBS. Sink conditions were maintained throughout the experiment. Methyl paraben was quantified using HPLC. The permeation of methyl paraben was evaluated by plotting the cumulative amount permeated per unit surface area of the membrane (µg/cm^2) against collection time in minutes.

Results and Discussion

Solubility of methyl paraben in each solvent

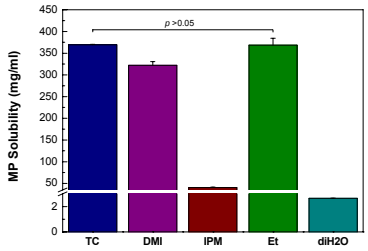


Figure 1. Solubility of methyl paraben in ■ TC, ■ DMI, ■ IPM, ■ Et and ■ deionised water (diH_2O) at 32°C. Error bars represent ±SD (n=3).

Amount of methyl paraben applied for finite dose studies

Formulations tested	Dose applied (mg/cm^2)
MP in IPM sat.	0.50
MP in DMI sat.	4.02
MP in TC sat.	4.62
MP in EtOH sat.	4.61
Neat Powder	4.60

Steady-state fluxes (infinite dose)

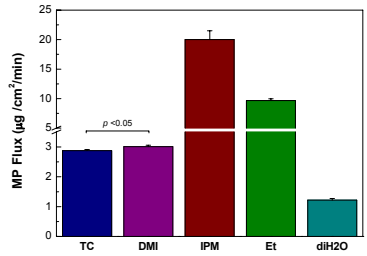
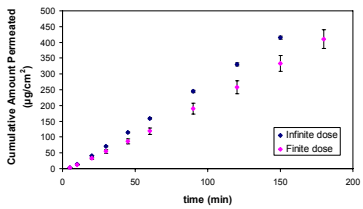


Figure 2. Estimated fluxes in µg/cm^2/min for the permeation of methyl paraben (infinite dose) in ■ TC, ■ DMI, ■ IPM, ■ Et and ■ water at 32°C. Fluxes were estimated from the slope of the steady-state portion of plots of cumulative amount permeated (in µg/cm^2) versus time in minutes. Error bars represent ±SD (n=5).

- ✓ The flux of MP from saturated solutions in IPM, DMI, TC and Et (infinite dose) across silicone membranes was higher than flux from aqueous solutions, suggesting solvent interaction with the membrane.
- ✓ Superior permeation enhancement was obtained using IPM, followed by Et, DMI and TC.

Finite dose vs. Infinite dose

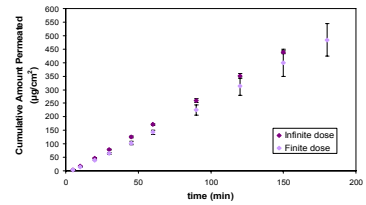
TC



Finite dose: ~10 % of the dose applied permeated after 3 hours

Figure 3. Cumulative amount of methyl paraben permeated across silicone membranes under infinite and finite dose conditions using TC as solvent at 32°C. Error bars represent ±SD (n=5).

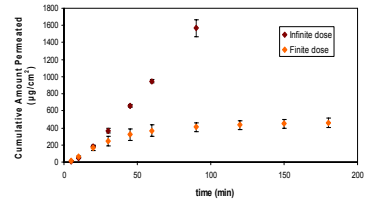
DMI



Finite dose: ~15 % of the dose applied permeated after 3 hours

Figure 4. Cumulative amount of methyl paraben permeated across silicone membranes under infinite and finite dose conditions using DMI as solvent at 32°C. Error bars represent ±SD (n=5).

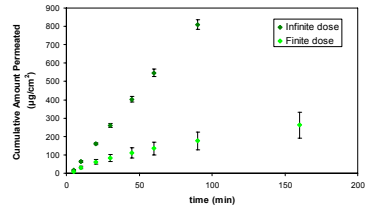
IPM



Finite dose: ~80 % of the dose applied permeated after 1.5 hours

Figure 5. Cumulative amount of methyl paraben permeated across silicone membranes under infinite and finite dose conditions using IPM as solvent at 32°C. Error bars represent ±SD (n=5).

Et

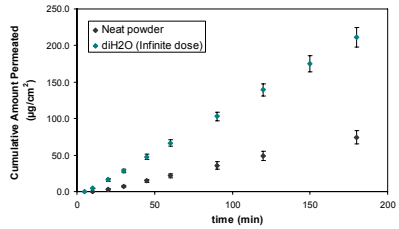


Finite dose: ~5 % of the dose applied permeated after 3 hours and ~20% after 24 hours (permeation rate decreased over time)

Figure 6. Cumulative amount of methyl paraben permeated across silicone membranes under infinite and finite dose conditions using Et as solvent at 32°C. Error bars represent ±SD (n=5).

- ✓ Drug crystals could be observed on the surface of the silicone membranes at the end of finite dose experiments using TC and DMI; in finite dose experiments using Et, drug in the donor completely crystallized ~5-10 min after application.
- ✓ Drug crystallization occurred because of solvent depletion in the donor, consequence of solvent evaporation and / or permeation into and out of the silicone membrane.
- ✓ Studies performed under infinite dose conditions overestimated the enhancement effect of both Et and TC. This was possibly because of solvent depletion from the donor following finite dose application, leading to drug crystallization and decreasing permeation.

Permeation of methyl paraben from neat powder



Neat powder: ~1.5 % of the dose applied permeated after 3 hours and ~10% after 24 hours (steady-state permeation)

Figure 8. Cumulative amount of methyl paraben permeated across silicone membranes from ♦ water (Infinite dose) and ♦ neat powder at 32°C. Error bars represent ±SD (n=5).

- ✓ The results show that permeation of methyl paraben in the absence of liquid solvent was possible (but quite low) under the experimental conditions used.
- ✓ The steady-state permeation rate was lower than using water as solvent (infinite dose) mainly because of reduced contact area of the powder with the membrane.

Conclusions

The overestimation of solvent enhancement effects under infinite dose conditions highlights the importance of using clinically relevant doses of applied formulation when conducting *in vitro* permeation studies, especially when significant depletion of the solvent from the formulation is expected. This work will be extended to human skin.

References

Trottet, L., Merly, C., Mirza, M., Hadgraft, J. and Davis, A. F. (2004). "Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride." International Journal of Pharmaceutics **274**: 213-219.

Acknowledgments

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