# Solvent Enhancement From Finite Dose Versus Infinite Dose In Human Skin

G. Oliveira, J. Hadgraft and M. E. Lane

Department of Pharmaceutics, The School of Pharmacy, University of London
29/39 Brunswick Square, London WC1N 1AX, United Kingdom

## Introduction and Aim

Historically, the most frequently used *in vitro* method for studying the effects of penetration enhancers on drug transport is the so called infinite dose technique. However, solvent evaporation and/or skin absorption of the vehicle may occur after application, which will ultimately impact on drug transport. Trottet et al. (2004) have shown that the depletion of propylene glycol from the formulation can limit its enhancing effect, especially when relatively small doses are used.

The aim of the present investigation was to study the effects of the vehicles commonly used in topical formulations, i.e. Transcutol P® (TC), dimethyl isosorbide (DMI), isopropyl myristate (IPM) and ethanol (EtOH), on skin permeation, when applied at clinically relevant doses. Methyl paraben was selected as a model compound, and saturated solutions were tested to ensure the same thermodynamic activity of the solute in all formulations.

## 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 ($diH_2O$). 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. *In vitro* diffusion experiments were conducted at 32 (±1)°C using Franz-type diffusion cells (1.13 $cm^2$ diffusion area) and PBS + 0.002% sodium azide as the receptor solution. Human epidermis prepared by the heat separation method (female Caucasian abdominal skin) was used. Finite dose studies were conducted by applying small volumes of the formulations (8.9 $\mu l.cm^{-2}$) which were evenly spread at the surface of the skin using a micropipette (non-occluded donor). Infinite dose studies were performed using 1ml of a saturated suspension of methyl paraben in each vehicle, i.e. containing undissolved drug to ensure maintenance of saturation during the experiment (occluded donor). Sampling occurred at designated time points with volume replacement using fresh receptor solution. 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 skin ($\mu mol/cm^2$) against collection time in hours.

## Results and Discussion

### Solubility of methyl paraben in each solvent

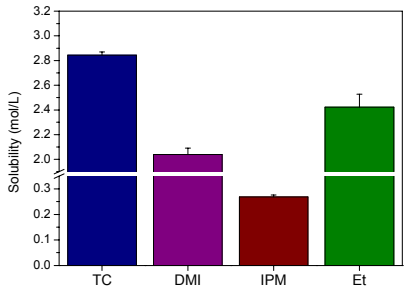



**Figure 1.** Solubility of methyl paraben in ■ TC, ■ DMI, ■ IPM and ■ Et at 32 C. Error bars represent ±SD (n=3).

✓ The solubility of methyl paraben was highest in TC, followed by Et, DMI and IPM ($p$ <0.05).

### Amount of methyl paraben applied for finite dose studies

| Formulations tested | Dose applied (μmol/cm²) |
|---|---|
| MP in IPM sat. | 2.0 |
| MP in DMI sat. | 19.1 |
| MP in TC sat. | 25.2 |
| MP in EtOH sat. | 20.9 |

### Permeation of methyl paraben under infinite dose conditions

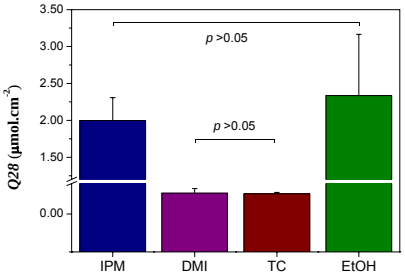
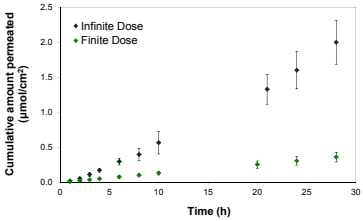



**Figure 2.** Cumulative amount of methyl paraben permeated after 28 hours (Q28 in μmol/cm²/min) across the skin under infinite dose conditions using ■ TC, ■ DMI, ■ IPM and ■ Et as vehicles. Error bars represent ±SD (n=4-5).

✓ Highest skin permeation of methyl paraben was obtained under infinite dose conditions using Et and IPM ($p$ >0.05), followed by both TC and DMI ($p$ >0.05).

### Finite dose vs. Infinite dose

**IPM**

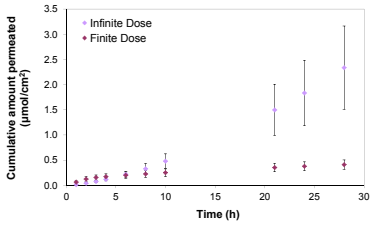



**Finite dose:** ~18 % of the dose applied permeated after 28 hours

**Figure 3.** Cumulative amount of methyl paraben permeated across skin under infinite and finite dose conditions using IPM as vehicle at 32 C. Error bars represent ±SD (n=5).

✓ Using IPM the permeation was higher under infinite dose compared with finite dose conditions, possibly because of the limited amount of vehicle applied (finite dose) which may limit the uptake/ interaction of IPM with the skin; hence its enhancing effect.

**Et**

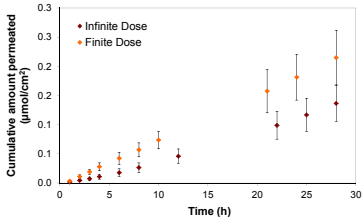



**Finite dose:** ~2 % after 28 hours (permeation rate decreased over time)

**Figure 6.** Cumulative amount of methyl paraben permeated across skin under infinite and finite dose conditions using Et as vehicle at 32 C. Error bars represent ±SD (n=4-5).

✓ Higher skin permeation was also obtained under infinite dose conditions using Et. This was because of the evaporation of the Et (i.e. solvent depletion) from the skin surface following finite dose application, leading to the crystallisation of methyl paraben and decreasing its availability to permeate.

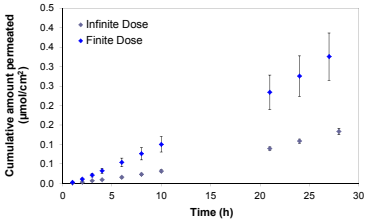
**DMI**




**Finite dose:** ~1.1 % of the dose applied permeated after 28 hours

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

**TC**




**Finite dose:** ~1.3 % of the dose applied permeated after 28 hours

**Figure 5.** Cumulative amount of methyl paraben permeated across skin under infinite and finite dose conditions using TC as vehicle at 32 C. Error bars represent ±SD (n=5).

✓ Interestingly, the skin permeation of methyl paraben was higher under finite dose conditions from both DMI and TC, compared with infinite dose conditions ($p$ <0.05). The reasons for this are not clear. However it is possible that the larger solvent volume used in the infinite dose study may promote vehicle-induced skin dehydration, thus increasing the barrier properties of the skin (Megrab, et al. 1995; Berner et al. 1989a, 1989b).

## Conclusions

The findings emphasise the importance of *in vitro* experimental design when testing topical formulations. Significant under estimation of the effects of permeation enhancers may occur when testing formulations under infinite dose conditions. *In vitro* testing of the effects of permeation enhancers should preferably be conducted under clinically relevant, finite dose conditions.

## References

Trottet, L. et al. (2004) Int. J. Pharm. 274: 213-219. Megrab, N.A. et al. (1995) J. Control. Release 36: 277-294. Berner, B. et al. (1989a) J. Pharm. Sci. 78: 402-407. Berner, B. et al. (1989b) J. Pharm. Sci. 78(6): 472-476

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