Method

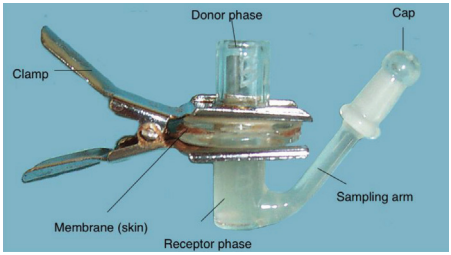
Preparation of test solution

5 different test solutions were prepared as shown in the table below:

Test solution	Treatment
A	In-use magnesium oil spray, dosed at 10 minutes interval up to 1 hour (5 doses), 11 µl per dose
B	Magnesium oil spray, 5 actuations (approx 1 ml)
C	31% w/v magnesium flakes in aloe vera juice + 0.2% w/v menthol
D	Saturated solution of magnesium flakes in deionized water
E	Deionized water, control

Preparation of porcine ear skin membrane

Pig ears obtained from the abattoir were cleaned under running water and the skin of the outer side of the ear removed by blunt dissection using a scalpel. The hairs were trimmed close to the skin using a pair of clippers, and the skin cut into sections of 2 x 2 cm, with care taken to choose the areas of the ear which were free from scarring or other noticeable defects which can compromise barrier integrity or provide resistance to permeating compounds.



In vitro skin permeation

The cut porcine ear skin sections were placed on pre-greased receptor compartment of glass Franz-type diffusion cells with the stratum corneum side facing upward (Figure). Micro magnetic stirring bars were added before hand to ensure complete distribution of permeating solutes in the receptor solution. The receptor solution was deionized water with 5% w/v gentamicin sulfate, and was degassed prior to use. The donor cells were pinch clamped on top, and the receptor compartment filled with temperature equilibrated receptor solution using a syringe. Complete cells were then placed on a magnetic stirring plate (Variomag, Daytona Beach, USA) in a water bath maintained at 37°C, providing a skin surface temperature of 32°C. Both the sampling arm and donor cells were occluded. Allowing 10 minutes for the cells to equilibrate with the receptor solution, the cells were then dosed with the test solutions.

The final volume of test solutions dosed on to the skin sections were as follows:

Test solution	Final volume dosed
A	11µl* x 5 doses = 55 µl (massaged)
B	5 pump actuations
C	11 µl
D	1 ml
E	11 µl

*Derived as follows: Holding the bottle 4 inch away, a sprayed area of 4.7cm diameter is achieved. This equates to ~17 square centimetres. Each spray delivers 0.185g solution. Therefore to replicate in-use conditions, we should add 0.185/17 = 0.011g (or 11 microlitres) per square centimetre. This was administered to the skin and massaged in using a blunt glass rod. Further doses were added at 10 minute intervals, in compliance with usage instructions.

Six replicates were prepared for each treatment, and the dosing area massaged using a glass rod with a gentle rotating motion. Entire receptor phases were removed using Pasteur pipettes at predetermined time points (3, 6, 12, 24, 36 & 48 hours) and replaced with temperature equilibrated receptor solution. The collected receptor phases were frozen in -20°C prior to analysis.

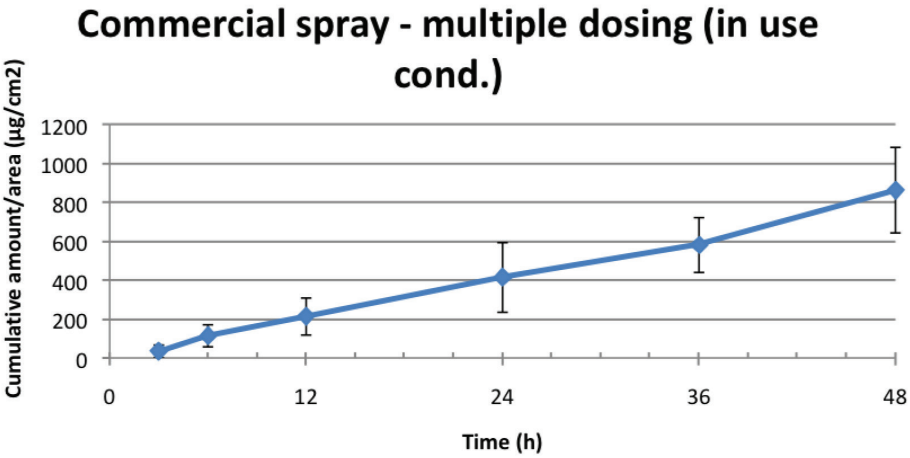
Inductively Coupled Plasma Mass Spectrometry (ICPMS)

The levels of magnesium were determined by ICPMS using a Thermo Elemental X Series 2 ICP-MS system equipped with a Plasma Screen. Analysis was performed in triplicate on each solution using 24Mg as the analytical mass. Calibration was carried out using synthetic standard solutions prepared from single element stock standards. Periodic checks for accuracy were performed by analysis of a solution of the international rock standard JB1a as an unknown. This standard was prepared by digesting a sample in HF/HNO3 and then HNO3 procedures described previously. Data was obtained as ppm and converted to mg/ml.

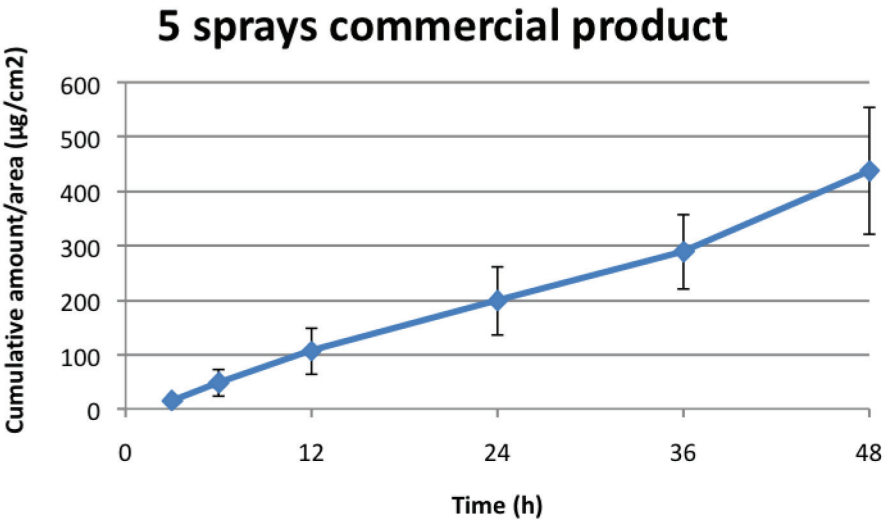
Cumulative mass of magnesium per area was plotted against time.

Results

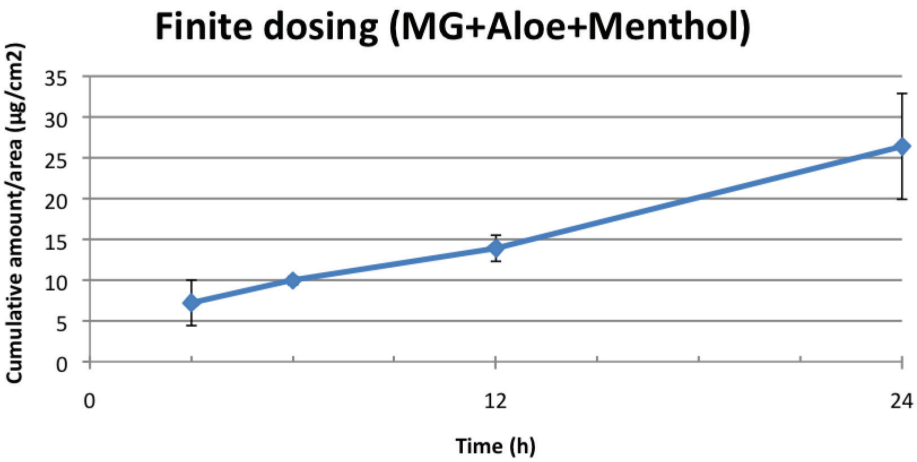
A. Magnesium oil, in use conditions



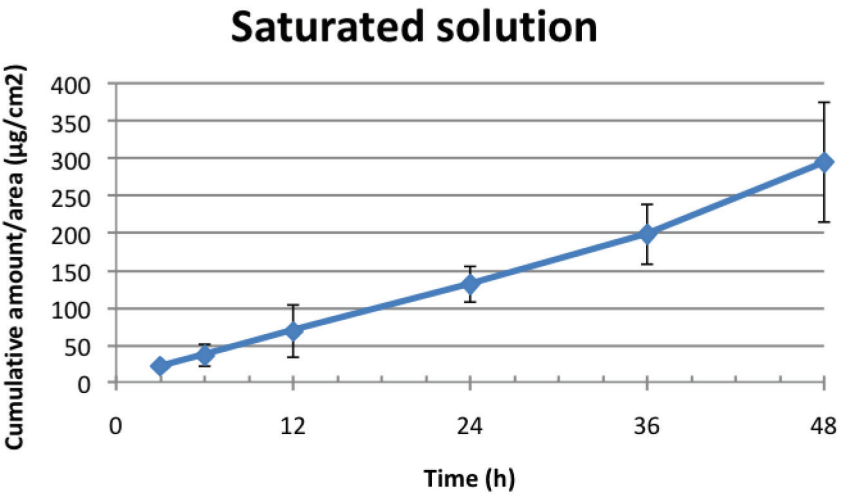
B. Magnesium oil, 5 sprays (1ml) Magnesium oil



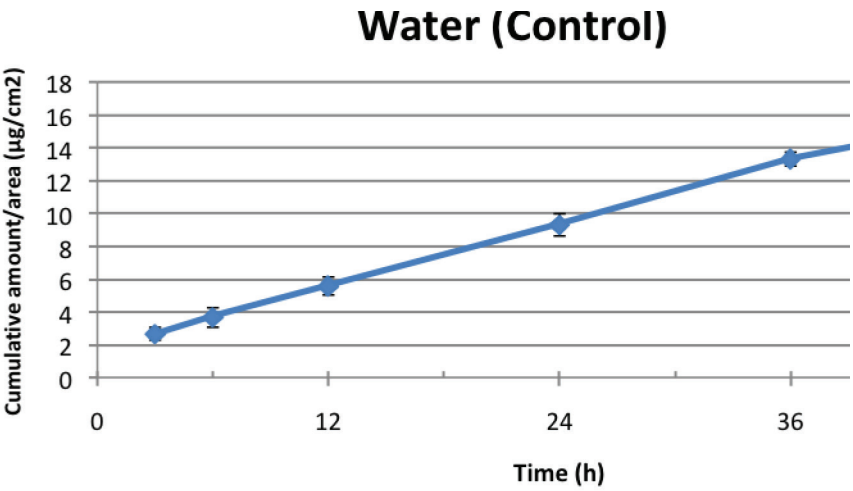
C. Model Magnesium oil, incorporating Aloe vera and menthol, in use



D. Saturated solution, ZECHSTEIN FLAKES in water

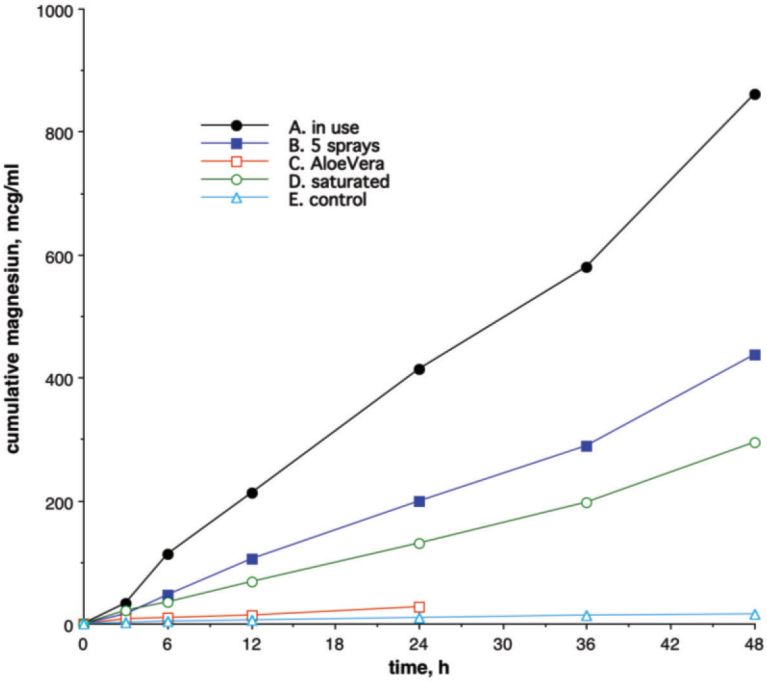


E. Water control



Overlaid plots

Cumulative mass



The greatest rate of permeation was observed with the current magnesium oil product, following an in-use protocol involving a sequence of finite doses, including massaging steps.

- The second greatest rate was obtained with the same product, dosed ‘infinitely’ as 5 shots, ie approx 1 ml.
- Next was the saturated solution of Zechstein MgCl2 flakes in water.
- Lastly, the reproduction Magnesium oil product based on Aloe instead of water and including menthol provided the lowest permeation.
- The control, skin dosed with water, illustrated that a relatively low amount of endogenous magnesium leached from the skin tissue over the course of the experiment.

Conclusions

Conclusions that can be drawn from the data:

1. Magnesium can permeate the skin from topically applied liquid solutions of MgCl2.
2. The level of magnesium that can be delivered appears maximal using the current Magnesium Oil product, following the stated instructions.
3. The act of massaging is key to achieving such a high dose.
4. The relatively low permeation of magnesium from the saturated solution was not expected. This may indicate other entities within the Magnesium Oil formulation.
5. The very low delivery of magnesium from 31% flakes in aloe vera plus menthol was unexpected. An explanation eludes us at present, although the presence of the components of Aloe vera could be promoting retention of magnesium within the skin.