NOTTINGHAM TRENT UNIVERSITY

In-vitro study of **amino acids** and **minerals** topological delivery

Studies auctioned by **NEO COSMETICS LIMITED**
April 8th, 2019

INTRODUCTION

Aim of study

To carry out skin permeation experiments on *Porcine* (pig) ears using Franz Cell apparatus and demonstrate systemic exposure of the client's proprietary transdermal PRE WORKOUT and POST WORKOUT formulations containing amino acids: arginine, glutamic acid, *L*-valine, *L*-leucine, *L*-isoleucine, lysine and glycine; and minerals: magnesium, calcium, sodium, potassium, zinc and iron.

Material and instrumentation used

NEOFIT products and all chemicals were used as received by the suppliers (NEO Cosmetics Limited, Sigma Aldrich, Acros and Alfa Aesar) unless stated otherwise. All other solvents were used as supplied (Laboratory, Analytical or HPLC grade), without further purification.

PerkinElmer Optima 5300 DV Inductively Coupled Plasma (ICP-OES), calibrated with Certipur® ICP Single-Element Standards.

Biochrom 30+ series Amino Acid Analyser, equipped with a lithium physiological prewash column, lithium high-resolution physiological column and a FD-500 Fluorescence detector, and calibrated with amino acid standard (Sigma-Aldrich AAS18).

METHOD

OECD protocol 428 *in-vitro* skin permeation to test transdermal formulations.

In-vitro skin permeation

Tissue samples suitable for Franz cell analysis were prepared, from pig ears obtained from abattoir on the day of analysis, by removing the outer side of the ear with a scalpel and cut into 1.5 cm dimeter disks. The tissues samples were stored in phosphate buffered saline (PBS), in an incubator (37^{o}C, 5 % CO_2) before mounting on to Franz cell.

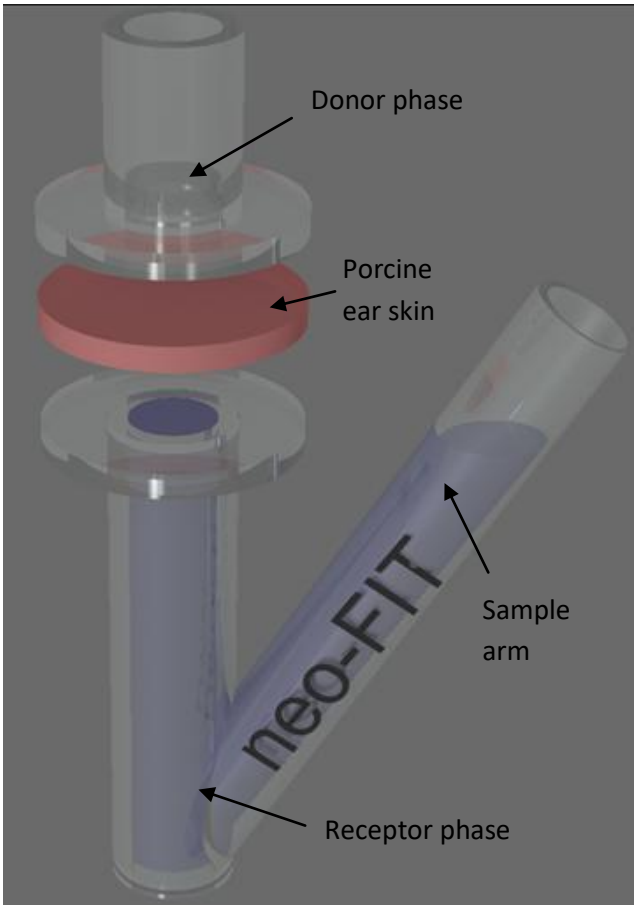


Figure 1: Franz cell

Freshly prepared porcine ear tissue samples where mounted into the Franz cell (as show in figure 1) with the stratum corneum side facing up and clamped into place. A Fisherbrand™ PTFE wing stir bar (5.5 mm diameter) was placed at the bottom of the Franz cell before filling with degassed reverse osmosis water (37 ^{o}C), ensuring there were no bubbles or voids between the tissue sample and the receptor phase. The sample arm was sealed using Parafilm®. The neo-FIT samples (0.1 mL) were applied as the donor phase to the exposed upper surface of the tissue sample (0.283 cm^2) and immediately massaged, with a gentle rotating motion, into the skin using a rounded glass rod, before being placed into an incubator (37^{o}C, 5% CO_2) equipped with a Thermo Scientific™ multipoint magnetic stirrer. Eight replicates were prepared for each NEOFIT formulation.

The Franz cell was removed from the incubator at scheduled intervals (10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 180 and 240 min). Once removed, an aliquot (1 mL) of the receptor phase was removed from the Franz cell using a needle and syringe and transferred to a centrifuge tube (2 mL – amino acid samples; 10 mL - mineral samples) and frozen (-78 ^{o}C) prior to analysis. An equivalent dose (1 mL) of degassed reverse osmosis water (37^{o}C) was added back into the Franz cell to make up the volume, ensuring there were no bubbles or voids between the tissue sample and the receptor phase, before returning to the incubator.

Elemental analysis *via* ICP-OES

The levels of trace minerals were determined by ICP-OES using a PerkinElmer Optima 5300 DV ICP-OES instrument. Analysis were

performed on each time point aliquot and quantified using a standard calibration from Certipur® ICP Single-Element standards of magnesium, calcium, sodium, zinc, iron and potassium.

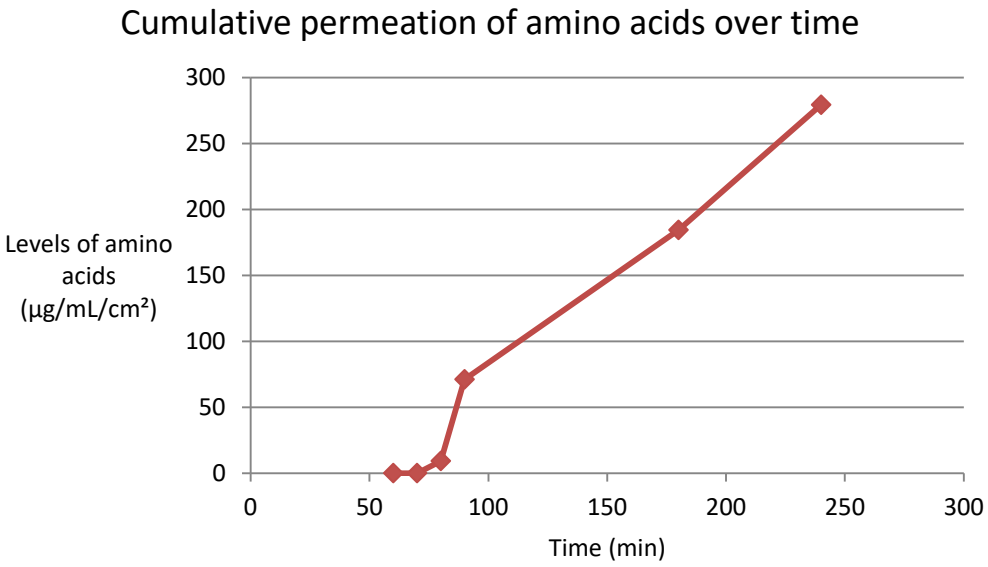
These samples were prepared by digesting the receptor phase aliquot (2mL) into 1% ultrapure HNO_3 (8 mL). Data was obtained as ppm and converted to mg/mL. Cumulative mass of trace minerals per area was plotted against time.

Amino acid analysis

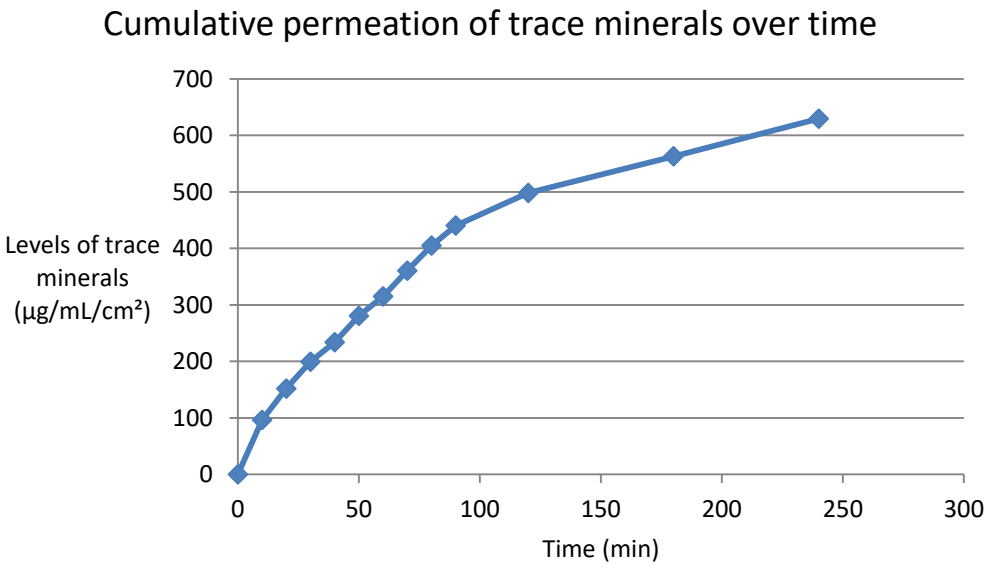
Amino acid concentrations in the receptor phase aliquots were determined using a BioChrom 30 Amino Acid Analyser. The amino acid standard (0.5 µmole/mL, except L-cysteine at 0.25 µmole/mL) was made up in 0.2 N sodium citrate, pH 2.2 and then diluted 1 in 10 from standard amino acid solution (30 µL into 270 µL buffer). The Franz cell samples were prepared by diluting the receptor phase (30 µL) into freshly prepared loading buffer (270 µL, pH 2.2) before loading into the BioChrom 30 auto sampler. The amino acid standard was repeatedly run every 5 samples to avoid any calibration drift.

RESULTS

PRE WORKOUT

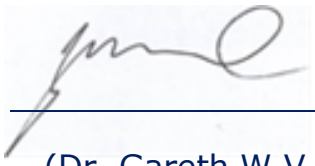


POST WORKOUT



CONCLUSION

1. The studies demonstrate that both topical formulations are transdermal and the amino acids and minerals cross the skin barrier.
2. (i) The delivery of amino acids commences after 80 minutes. This is likely due to the fact amino acids are too large to pass through ion channels and are not sufficiently lipophilic to penetrate through lipid membranes, they must therefore employ specific uptake *via* sodium-dependent amino acid transporters.
   (ii) Delivery of amino acids over 4 hours is indicative of re-enforcing muscle feed, thus helping to prevent muscle fatigue and sustain performance.
3. Free minerals are dissociated into smaller ions, therefore crossing the skin barrier upon application, which is ideal for faster recovery times post training.
4. The plotted results clearly show tests over a longer period of time are warranted as it seems probable there is a continuous release of amino acids and minerals to muscles beyond 4 hours.

Signed:  Date: 8th April 2019

(Dr. Gareth W.V. Cave)