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RESEARCH PAPER

In Vitro–In Vivo Correlation in Skin Permeation

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ABSTRACT

Purpose *In vitro* skin permeation studies have been used extensively in the development and optimisation of delivery of actives *in vivo*. However, there are few reported correlations of such *in vitro* studies with *in vivo* data. The aim of this study was to investigate the skin permeation of a model active, niacinamide, both *in vitro* and *in vivo*.

Methods Conventional diffusion cell studies were conducted in human skin to determine niacinamide permeation from a range of vehicles which included dimethyl isosorbide (DMI), propylene glycol (PG), propylene glycol monolaurate (PGML), N-methyl 2-pyrrolidone (NMP), Miglyol 812N® (MG), and mineral oil (MO). Single, binary or ternary systems were examined. The same vehicles were subsequently examined to investigate niacinamide delivery *in vivo*. For this proof-of-concept study one donor was used for the *in vitro studies* and one volunteer for the *in vivo* investigations to minimise biovariability. Analysis of *in vitro* samples was conducted using HPLC and *in vivo* uptake of niacinamide was evaluated using Confocal Raman spectroscopy (CRS).

Results The amount of niacinamide permeated through skin *in vitro* was linearly proportional to the intensity of the niacinamide signal determined in the stratum corneum *in vivo*. A good correlation was observed between the signal intensities of selected vehicles and niacinamide signal intensity.

Conclusions The findings provide further support for the use of CRS to monitor drug delivery into and across the skin. In addition, the results highlight the critical role of the vehicle and its disposition in skin for effective dermal delivery.

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INTRODUCTION

Delivery of a drug or cosmetic active into and through the skin involves a number of steps including release from the vehicle, partition into the skin and subsequent movement through the stratum corneum. The amount of an active ingredient that can permeate through the skin will also be influenced by factors such as the integrity of the skin barrier, the physico-chemical properties of the permeant as well as those of the vehicle components (excipients). Ideally, the disposition of an active after application to the skin should be measured by *in vivo* experiments on humans. However, such studies are expensive, time-consuming and have ethical implications. In addition mass balance studies are difficult to conduct *in vivo* without taking biopsies (or tape-stripping), which are invasive (or minimally invasive). For these reasons, there has been a reliance for many years on *in vitro* skin permeation studies for assessment of the skin penetration of drugs from topical or transdermal products. Typically, excised human skin is mounted in a diffusion cell with the membrane being clamped between donor and receptor chambers.

Franz and co-workers have reported on the correlation of *in vitro* studies with *in vivo* absorption for a range of molecules (1). *In vitro* skin permeation data for seven approved generic topical drug products were compared with corresponding reference products. Clinical data for the same products were obtained following their regulatory approval. For six of the seven products the ratio of the *in vitro* test to the reference was close to unity, indicating that the products were equivalent which was in agreement with the clinical data. More recently, the same group reviewed 92 percutaneous absorption data sets collated from 30 published studies and calculated an *in vitro - in vivo* correlation using the percentage of applied dose

permeated as the metric for comparison (2). When the data were normalised, the average *in vitro - in vivo* correlation (IVIVC) ratio was 0.96 and there was a less than a two-fold difference between the *in vitro* and *in vivo* results for any one compound.

Confocal Raman Spectroscopy (CRS) has been used to analyse the thickness of the SC in humans *in vivo* by combining the principle of confocal microscopy with Raman spectroscopy within the skin in real-time (3,4). Recently we have reported the application of CRS to obtain profiles of ibuprofen in skin *in vivo* (5). The results compared very well with similar data collated from earlier tape-stripping studies and suggest that CRS offers considerable potential for profiling drug disposition in skin following topical application.

In the present study we evaluated the *in vitro* permeation of the model permeant niacinamide (Fig. 1) from seven different vehicles and compared the *in vitro* permeation data with the results obtained *in vivo* using CRS. In order to minimise biovariability in this proof-of-concept study *in vitro* studies were conducted on skin from one donor and *in vivo* spectroscopic datat were collected from one human subject.

Niacinamide was selected as a model permeant because it has been used in pharmaceutical and cosmetic formulations for many years. It is also reported to provide several beneficial effects to aging skin by improving barrier function, decreasing signs of photoaging and reducing sebum production (6–8). The physicochemical properties of niacinamide are summarised in Table I.

The vehicles selected for evaluation of niacinamide delivery encompass a range of physicochemical properties and have been investigated extensively for topical delivery of actives. Single, binary and ternary systems of the following solvents were examined: dimethyl isosorbide (DMI), Miglyol 812N® (MG), mineral oil (MO), propylene glycol (PG), propylene glycol monolaurate (PGML) and N-methyl 2-pyrrolidone (NMP).

Table I Physicochemical Properties of Niacinamide

Molar mass[a]	122.12 g/mol^{-1}
Log P[a]	−0.4
Melting point[a]	128–131°C
Aqueous solubility[b]	>750 mg ml^{-1}
Solubility parameter[c]	13.9 (cal/cm^3)$^{1/2}$
pK_a[a]	3.3 (20°C)

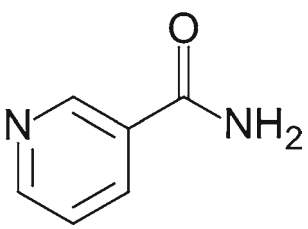
[a] From reference (9)
[b] From reference (10)
[c] Calculated using Molecular Modelling Pro™ (ChemSW, Fairfield, CA, USA)

MATERIALS AND METHODS

Materials

Niacinamide was a generous gift from Procter & Gamble (Egham, U.K.). Phosphate buffer saline (PBS) tablets were obtained from Oxoid Ltd (Basingstoke, UK). Deionised water was prepared using an Elgastat water purifier (Elga Ltd. Buckinghamshire, U.K.). Phosphoric acid was purchased from BDH (U.K.) and diethylamine and NMP were obtained from Fisher (U.K.). An electronic balance (Sartorius, Epsom, U.K., ± 0.0001 g accuracy) was used to weigh all materials. DMI was a gift from Croda Ltd (Snaith, Goole, U.K.), PG was obtained from Sigma-Aldrich (Germany), Miglyol 812N® from Sasol GmbH (Germany), PGML was a gift from Gattefossé (Saint Priest, France) and MO was purchased from J.M. Loveridge Ltd (U.K.). Full-thickness Caucasian female abdominal human skin from one donor following cosmetic surgery was obtained with informed consent and appropriate institutional ethical approval. The epidermis was separated by the heat separation method as reported previously (11) and stored at −20°C until required.

Fig. 1 Chemical structure of niacinamide.



Solubility and Solubility Parameter Determination

Solubility measurements were conducted as reported previously (12) at 32 ± 1°C and samples were analysed using HPLC (High-Performance Liquid Chromatography (HPLC)). The solubility parameters of the solvents and binary and ternary systems (Table II) were calculated with Molecular Modelling Pro™ (ChemSW, Fairfield, CA, USA) using the Van Krevelen and Hoftyzer approach (13).

High-Performance Liquid Chromatography (HPLC)

The HPLC system used consisted of a HP series 1100 quaternary pump, HP 1100 autosampler, HP series 1100 system controller, HP series 1100 degasser and HP series 1100 diode array detector. The data were acquired and analysed using PC ChemStation® for LC 3D (Rev.A.09. 03, Agilent Technologies, U.S.A). A Luna Phenyl-Hexyl column, 250×4.60 mm, 5 µm (Phenomenex, U.K.) was used with a mobile phase consisting of methanol:water (80:20). The retention time was 4.5 min at a wavelength of 263 nm. The specificity of assays was assessed by analysis of at least five blank samples. Calibration curves were constructed on the basis of peak area measurements. Reproducibility was evaluated and coefficients of variance were < 5% in all experiments.

Table II Vehicle Solubility Parameter and Niacinamide Solubility at 32°C ($n = 3$; Mean ± S.D.)

Vehicle	Solubility parameter $(cal/cm^3)^{1/2}$	Niacinamide solubility (% w/v)
DMI	10.0	7.4 ± 0.1
MG	7.8	0.3 ± 0.04
MO	7.7	$3 \times 10^{-4} \pm 2 \times 10^{-5}$
NMP	10.8	45.2 ± 0.8
PG	14.1	31.2 ± 0.6
PGML	9.4	3.3 ± 0.1
DMI:MG (90:10)	9.8	10.8 ± 0.1
NMP:MG (50:50)	9.3	6.2 ± 0.7
PG:PGML (50:50)	11.8	24.6 ± 0.6
DMI:MG:MO (75:22.5:2.5)	9.4	8.6 ± 0.4
NMP:MG:MO (40:50:10)	9.0	5.4 ± 0.2
PG:PGML:MG (20:50:30)	9.9	13.5 ± 0.4

In Vitro Skin Permeation Experiments

In vitro human skin permeation experiments were conducted as reported previously using vertical glass Franz diffusion cells (14). Solutions of niacinamide (5% w/v) were prepared in three single solvents PG, DMI, NMP, three binary systems PG:PGML, DMI:MG, NMP:MG, and three ternary systems PG:PGML:MG, DMI:MG:MO, NMP:MG:MO. This concentration of niacinamide was chosen as it is typically used in personal care products at this level. The proportions of each solvent in the binary and ternary systems are detailed in Table II. An infinite dose of each formulation (1 ml/cm^2) was introduced into the donor chamber. The Franz cells used had a mean diffusion area of 1.14 ± 0.12 cm^2 and a receptor volume of 2.11 ± 0.12 ml. The donor and receptor compartments were occluded using glass cover lids and Parafilm® to prevent evaporation. The experiment was conducted over 48 h at 32 ± 1°C and the number of replicates was at least four. At appropriate time intervals, 200 µl from the receptor phase was withdrawn and immediately replaced by an equal volume of fresh receptor fluid (PBS, pH 7.3 ± 0.1). In the data analysis allowance was made for the amount removed and the dilution as a result of addition of fresh receptor phase. The values of flux were obtained by plotting the cumulative amount of niacinamide in the receptor phase as a function of time. Linear regression analysis (Excel, Microsoft Office 2007) was used to achieve the gradient, which is equivalent to the flux.

Solvent Uptake into the Skin

In order to probe any correlation between solvent uptake and delivery of active to the skin from specific vehicles solvent uptake studies were conducted. The skin was placed in desiccators for 24 h before it was cut into small pieces (10.2 ± 4.0 mg). The uptake of different formulations into skin was determined using a gravimetric method. The membranes were individually soaked in an excess of the formulation (2 ml) in sealed glass vials which were placed in a temperature controlled oven (Jouan, Medical Supply Co., Boxtel, Netherlands) at 32 ± 1°C for 48 h. The membranes were then removed from the solvent, blotted dry with tissue paper and reweighed. A similar protocol has been used previously to examine solvent uptake by silicone membrane or human skin (11,15). The 48 h period for the experiment was selected as the *in vitro* permeation study was run for a maximum of 48 h. In addition, initial experiments indicated that no further mass uptake occurred after 48 h indicating that the system had reached equilibrium. The number of replicates was five, and the amount of solvent taken up by the membrane was expressed as a percentage mass increase.

In Vivo Studies - Confocal Raman Spectroscopy

The *in vivo* CRS measurements were carried out using a River Diagnostics 3510 Skin Analyser (River Diagnostics, Rotterdam, The Netherlands) with appropriate institutional ethical approval (NHS REC 10/H0801/69). The device comprises a high-performance dispersive spectrometer with 671 nm and 785 nm laser excitation and a confocal measurement stage. A fingerprint region of the skin was obtained using the 785 nm wavelength (a 1 µm spot of ≤ 20 mW power). A measurement capture time of 10 s per point within the scan was selected. Scans of 4 µm steps over a total depth of 40 µm into the tissue were collected. The intensity of the signals for niacinamide and selected vehicles were measured from these fingerprint region spectra and expressed in arbitrary units. A total of eight scans were performed for each measurement site, at each time point, by positioning the laser on a different skin area within the same site. The data were analysed using SkinTools v.2.0 (River Diagnostics, Rotterdam, The Netherlands). The effect of various solvents or formulations on the permeation of niacinamide was investigated by applying 5% w/v niacinamide in the various vehicles to the volar forearm of the investigator for 30 min following a previously published protocol (16). The dose applied was 2 mg/cm^2 and only one volunteer was selected so that biological variability was minimised. This was important as the objective of this proof-of-concept study was to determine the feasibility of CRS as an appropriate technique to assess bioequivalence in skin and its correlation with *in vitro* permeation studies. In addition, the *in vitro* permeation studies were conducted on skin from one donor.

Data Analysis

SPSS version 18 and Microsoft® Excel Office 2007 were used to analyse the data. Normality was assessed using the Kolmogorov-

Smirnov statistical test. Parametric statistical tests (one-way between group analysis of variance, ANOVA and paired *t*-test to compare means) were used to investigate statistical differences. A probability of $p<0.05$ was considered statistically significant. All results are presented as mean and standard deviation.

RESULTS AND DISCUSSION

Solubility and Solubility Parameter

The solubility of niacinamide in each vehicle is shown in Table II with their respective solubility parameters.

The solubility parameter of niacinamide was calculated as 13.85 $(cal/cm^3)^{1/2}$ as reported in Table I. High solubility is evident in PG, which is not surprising considering the proximity of the respective solubility parameters of niacinamide and this solvent. The high solubility of NMP is notable and may reflect the ability of NMP to form hydrogen bonds with niacinamide and hence form a solvent cage (17).

Permeation Studies

The flux, lag time and permeability coefficient values of niacinamide for all vehicles are reported in Table III.

The highest flux values were observed for the PG:PGML:MG formulation and these were significantly larger ($p<0.05$) than the values for the NMP:MG:MO, NMP:MG and PG:PGML vehicles. In general, those formulations that contained PG or NMP were associated with higher flux values compared with those containing DMI. The NMP vehicles had significantly lower t_{lag} values ($p<0.05$) compared with the other formulations, while the highest t_{lag} value was observed for the PG:PGML:MG (paired *t*-Test, $p<0.05$). The highest k_p value was also observed for the PG:PGML:MG vehicle.

Table III Steady State Flux, t_{lag} and Permeability Coefficients of Niacinamide in Skin at 32°C ($n\geq4$; Mean ± SD)

Vehicle	J ($\mu g/cm^2.hr$)	t_{lag} (h)	k_p (cm/hr)
PG	0.22 ± 0.06	5.13 ± 1.98	4.45E-06 ± 1.22E-06
DMI	0.11 ± 0.06	8.89 ± 0.42	2.26E-06 ± 1.12E-06
NMP	45.07 ± 1.43	0.24 ± 0.11	9.01E-04 ± 2.87E-05
PG:PGML	96.10 ± 41.43	0.56 ± 0.31	1.92E-03 ± 8.29E-04
DMI:MG	8.97 ± 2.85	10.45 ± 4.68	1.79E-04 ± 5.69E-05
NMP:MG	120.99 ± 15.04	0.40 ± 0.11	2.42E-03 ± 3.01E-04
PG::PGML:MG	187.00 ± 39.06	16.17 ± 2.63	3.74E-03 ± 7.81E-04
DMI:MG:MO	1.58 ± 0.78	12.04 ± 5.03	2.39E-05 ± 3.50E-06
NMP: MG: MO	125.01 ± 31.96	0.26 ± 0.33	2.50E-03 ± 6.39E-04

Solvent Uptake

Liron and Cohen (18) estimated the solubility parameter of human skin to be 10 $(cal/cm^3)^{1/2}$. It may be hypothesised that if the solubility parameter of the vehicle alters that of the skin so that it is closer to the solubility parameter of the drug, permeation may be enhanced. We have previously investigated the interplay between solubility parameter of solvents and permeants with reference to membrane diffusion of the permeant (11,12). In the present work, solvent uptake studies were conducted to determine any relationship between solubility parameter of the vehicle and skin uptake and the results are reported in Table IV.

The highest percentage of vehicle uptake into the skin was noted for PG:PGML:MG followed by DMI and DMI:MG. This reflects the proximity in solubility parameter values for these formulations to that reported for skin and is consistent with the hypothesis that vehicles with solubility parameter values closest to that of skin will be well taken up by this membrane.

Confocal Raman Studies

The profiles for the niacinamide signal intensity up to 20 μm (Fig. 2) were calculated based on the spectra obtained from the formulations with and without 5% w/v niacinamide.

It is noteworthy that the vehicle which was taken up to the greatest extent in the *in vitro* studies (Table IV) is in fact the vehicle which promotes the highest relative delivery of niacinamide to skin *in vivo*. However, there is no discernible trend for solvent uptake into skin *in vitro* and delivery of the active *in vivo* for the range of vehicles studied.

The data in the figures show important findings which firstly, have the potential to lead to new approaches to measure bioequivalence in skin and secondly, should allow optimisation of formulations to delivery active agents to the skin. There are clear differences between the different solvents. In one particular instance, the ternary system, it is clear that the concentration depth profile cannot be modelled simply by a

Table IV Vehicle Solubility Parameters and Uptake by Human Skin at 32°C ($n\geq4$; Mean ± S.D.)

Vehicle	Solubility parameter $(cal/cm^3)^{1/2}$	% increase in skin weight
PG	14.1	23.1 ± 7.2
DMI	10.0	81.5 ± 12.8
PG:PGML	11.8	34.0 ± 5.2
DMI:MG	9.8	68.3 ± 9.0
PG:PGML:MG	9.9	99.2 ± 21.4
DMI:MG:MO	9.4	32.7 ± 15.9
NMP:MG:MO	9.0	14.3 ± 3.0

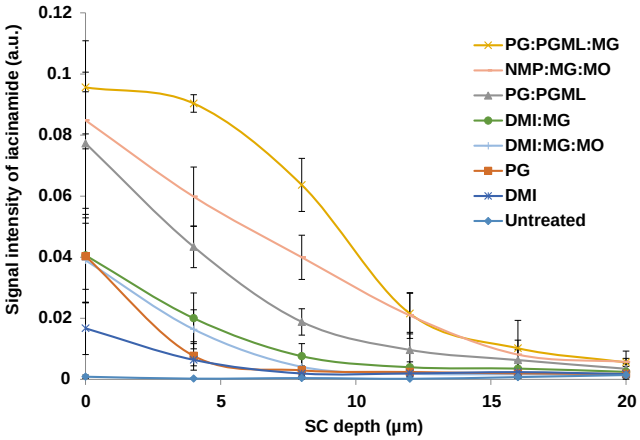


Fig. 2 Total signal intensity of niacinamide permeated through the mid ventral forearm up to 20 μm after a 30 min application of each vehicle ($n = 8$: Mean ± S.D.).

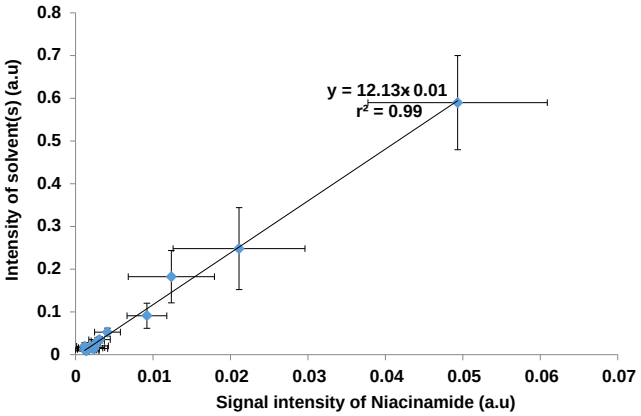


Fig. 4 Correlation between signal intensity of niacinamide and signal intensity of PG or DMI which permeated into the SC *in vivo* with depth (0, 4, 8, 12, 16, 20 μm into the SC) for the mid ventral forearm of the investigator, ($n = 8$; Mean ± S.D.).

solution to Fick's second law of diffusion. The solvents penetrate the skin and modify the barrier properties such that the expected exponential decrease in concentration with depth is not observed. With appropriate software it should be possible to fit the data to transport equations and show the impact that the presence of the solvent has on the partition and diffusion properties of the different layers in the skin. This may be dependent on both time after application of the solvent (s) and depth of penetration into the SC. When the *in vitro* skin permeation data are plotted against the signal intensity for niacinamide *in vivo*, expressed in arbitrary units, at a depth of 4 μm, a linear correlation is obtained (Fig. 3a).

Figure 3a shows clearly that there is a correlation between *in vitro* experiments conducted on human skin and *in vivo* data collected by CRS. Figure 3b illustrates the same correlation for low flux values. To investigate further, the intensity of the niacinamide signal after the 30 min application period was also plotted against the amounts of selected vehicles (PG and DMI) measured by CRS, at different depths (Fig. 4).

Figure 4 shows the impact that these simple solvents have on the uptake of the permeant and, in this case, there was also an excellent agreement between the amount of solvent in the skin and the signal intensity for niacinamide uptake into the SC.

CONCLUSIONS

The solvents investigated in this proof-of-concept study have been investigated extensively for their ability to promote topical and transdermal delivery of a range of molecules (19–23). However, the mechanisms underlying their ability to modulate

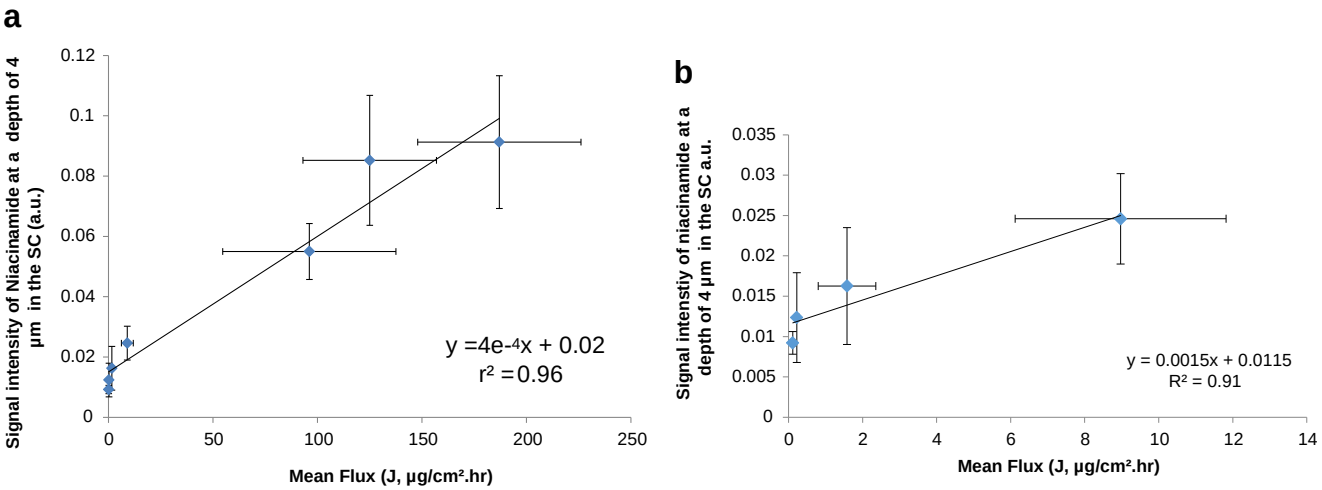


Fig. 3 (**a**) Correlation of the *in vitro* flux of niacinamide and signal intensity of niacinamide permeated into the mid ventral forearm at 4 μm *in vivo* after a 30 min application of each vehicle ($n = 8$; Mean ± S.D.) (**b**) Correlation of the *in vitro* flux of niacinamide and signal intensity of niacinamide permeated into the mid ventral forearm at 4 μm *in vivo* after a 30 min application of each vehicle for flux values up to 10 mg/cm2 ($n = 8$; Mean ± S.D.).

the permeation of actives have not been well explored. Solvent uptake studies provide only limited insight into formulation effects as the formulation with the highest uptake, PG:PGML:MG, also resulted in comparatively high skin flux values; on the other hand DMI was also absorbed well by the skin but had little apparent enhancement effects on niacinamide skin permeation *in vitro* and *in vivo*. These vehicles also had solubility parameter values closest to the value estimated for human skin. Uptake and interaction of the individual formulation components with the skin is expected to change the partitioning of the drug into, and/or diffusion across the membrane (24). Understanding how combinations of skin penetration enhancers will modulate these effects remains a challenging task for formulation scientists.

CRS has previously been used to monitor the penetration of dimethyl sulphoxide from a propyelene glycol/water vehicle (25). In the present study, monitoring of the vehicle as well as the active was conducted but was limited to single solvents which exhibited a suitable Raman signal. It was possible to examine the signal intensity for the active which permeated *in vivo* for all formulations. For this preliminary study intended to demonstrate the feasibility of CRS in assessing drug delivery from topical formulations, the *in vivo* data correlated very well with *in vitro* skin permeation data.

For the PG and DMI vehicles a positive correlation between the signal intensity of niacinamide in skin and the vehicle uptake into skin was obtained. Similar findings have been observed for *in vitro* skin permeation studies. For example, Berner *et al.* reported a linear correlation between the flux of nitroglycerin across the skin and ethanol (26). Trottet *et al.* noted that the skin permeation of loperamide *in vitro* was dependent on the amount of propylene glycol used in the vehicle (27).

Clearly CRS offers a number of advantages for studying topical drug delivery compared with conventional diffusion experiments. Using CRS, real time *in vivo* signal intensity data of the active were collected and experiments were conducted over 1 h compared with 48 h for the *in vitro* permeation studies. The time course over which the CRS measurements were conducted is very much shorter than the *in vitro* experiments and such *in vivo* studies should facilitate the optimisation of formulation development since they can be conducted *in vivo* over a small area of skin and over a short time. This proof-of-concept study reported *in vitro* permeation data from one donor and *in vivo* results from one subject; future studies will expand the analysis to a larger pool of donors and samples. Work is also currently underway to investigate if the *in vitro* - *in vivo* correlation observed here may be applicable to other molecules.

CRS is an important tool in the current armament that skin biophysicists have to interrogate the properties of the skin. Based on the preliminary data reported here we believe that it should be possible to develop the technique to show the impact of the formulation on the barrier properties of the skin. This in turn will allow the generation of formulation optimisation possibilities and permit the generation on a non-invasive means of assessing skin bioequivalence.

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