

***In vitro* permeation and disposition of niacinamide in silicone and porcine skin of skin barrier-mimetic formulations**

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ABSTRACT

Niacinamide (NIA) is an amide form of vitamin B3 which is used in cosmetic formulations to improve various skin conditions and it has also been shown to increase stratum corneum thickness following repeated application. In this study, three doses (5, 20 and 50 μl per cm^2) of two NIA containing oil-in-water skin barrier-mimetic formulations were evaluated in silicone membrane and porcine ear skin and compared with a commercial control formulation. Permeation studies were conducted over 24 h in Franz cells and at the end of the experiment membranes were washed and niacinamide was extracted. For the three doses, retention or deposition of NIA was generally higher in porcine skin compared with silicone membrane, consistent with the hydrophilic nature of the active. Despite the control containing a higher amount of active, comparable amounts of NIA were deposited in skin for all formulations for all doses; total skin absorption values (permeation and retention) of NIA were also comparable across all formulations. For infinite (50 μL) and finite (5 μL) doses the absolute permeation of NIA from the control formulation was significantly higher in porcine skin compared with both test formulations. This likely reflects differences in formulation components and/or presence of skin penetration enhancers in the formulations. Higher permeation for the 50 and 20 μl dose was also evident in porcine skin compared with silicone membrane but the opposite is the case for the finite dose. The findings point to the critical importance of dose when evaluating topical formulations *in vitro* and also the likelihood of exaggerated effects of excipients on permeation at infinite and pseudo-finite dose applications.

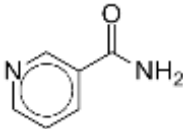
Keywords: Niacinamide, Franz diffusion cell, porcine skin, silicone membrane, barrier-mimetic, formulation

1. Introduction

Following topical application, the amide form of vitamin B3, niacinamide (NIA), has been found to improve several dermatological conditions including acne, atopic dermatitis, aging of skin and ultraviolet-induced DNA damage [1,2]. NIA has also been shown to enhance skin barrier function [3,4], reduce pore size, improve skin texture [5], exert beneficial effects in an autoimmune blistering disorder [1], and improve the appearance of wrinkles blotchiness and hyper pigmented skin areas [6]. In 2005, the Cosmetic Ingredient Review panel published a report on the safety assessment of NIA noting that the active was used in more than 60 cosmetic formulations [7]. However, there are >500 products currently in use listed as containing NIA in the Environmental Working Group (EWG) Skin Deep® Cosmetic Database [8]. NIA is largely found in shampoos, skin moisturisers and cleansing formulations [8]. Amounts of NIA ranging from 2-5% are used in products to even out skin colour and tone, enhance skin barrier function, and decrease skin sensitivity to surfactants [4]. Products containing NIA at 3% and 4% are used in body and hand creams and preparations to treat acne respectively [7,8].

NIA has a low molecular weight and is a water soluble compound (Table 1) and therefore does not possess the ideal physicochemical properties for skin delivery. The aim of the present study was to examine the skin permeation and disposition of NIA from two skin barrier-mimetic formulations compared with a commercial formulation. A secondary aim was to examine how the dose of formulation applied influenced permeation in an artificial membrane and porcine skin.

Table 1. Physicochemical properties of NIA

Molecular structure	
Molecular weight*	122.1 Da
Melting point [9]	130°C
Boiling range [7]	150-160°C
Log of partition coefficient (log P)*	-0.35
Solubility parameter [10]	13.9 (cal/cm ³) ^{1/2}
pK _a [9]	3.35 (20°C)
Density [8]	1.40

*Calculated with ChemBioDraw®

2. Materials and methods

2.1 Materials

NIA was purchased from Sigma Aldrich, UK. A commercial oil-in-water control formulation containing niacinamide was purchased from a retail outlet. The formulation contained the following sunscreens: ethyl hexylsalicylate, butyl methoxydibenzoylmethane, octocrylene, phenylbenzimidazole sulfonic acid, zinc oxide, titanium dioxide and benzyl salicylate.

Two test formulations (Formulation A and B) were supplied by Glaxo Smith Kline (GSK, UK). Formulation A contained hydrogenated lecithin, capric caprylic triglyceride, isoamyl p-methoxycinnamate, diethylamino hydroxybenzoyl hexyl benzoate, bis-ethylhexylphenol methoxyphenyl triazine, shea butter, glycerine, olus oil, isostearyl isostearate, dicapryl carbonate, xylitol, panthenol, niacinamide, pentylene glycol and 1,2 hexanediol. Formulation B contained hydrogenated lecithin, capric caprylic triglyceride, shea butter, glycerine, olus oil, isostearyl isostearate, dicapryl carbonate, xylitol, panthenol, niacinamide, pentylene glycol and 1,2 hexanediol. HPLC grade water, methanol, orthophosphoric acid, diethylamine, formic acid and acetonitrile were obtained from Fisher Scientific, UK. Phosphate buffer saline (PBS) tablets were purchased from Oxoid Limited, England. Silicone membrane (polydimethylsiloxane, 80 μm thickness) was a kind donation from Dow Corning (Seneffe, Belgium).

2.2 High performance liquid chromatographic (HPLC) analysis

NIA analysis was conducted using a HPLC (Agilent Technologies 1200 series) equipped with an Agilent G1322A degasser, G1311A quaternary pump, G1329A auto sampler and G1316A thermostat column compartment. Analysis was performed using a Phenomenex Luna Phenyl Hexyl column fitted with a guard column. The length, internal diameter and particle size were 250 mm, 4.6 mm and 5 μm , respectively. The mobile phase consisted of water:methanol (80:20). The pH of the mobile phase was adjusted to 7.0 ± 0.2 using orthophosphoric acid and diethylamine. The mobile phase was degassed using an ultrasonicator (VWR International) prior to use to remove air bubbles. The flow rate of the mobile phase was 1 mL/min and the column temperature was set at 40°C. The chromatogram was acquired at a wavelength of 263 nm. A sample volume of 10 μL was injected for a total run time of 10 min. A known amount of NIA was dissolved in PBS (pH 7.3 ± 0.1) and a stock solution (500 $\mu\text{g}/\text{mL}$) was prepared. The stock solution was diluted to prepare various concentrations of NIA. The NIA peak was evident at 4.8 min. The calibration curve was constructed in the concentration range of 0.5 to 50 $\mu\text{g}/\text{mL}$. A linear relationship was found between concentration and peak area with regression coefficient values (r^2) of greater than 0.99. The

accuracy value of the developed method was $100\pm 1.25\%$. The relative standard deviation (RSD) values of intra and inter day precision values were 0.37 and 3.20%, respectively.

2.3 Solubility determination

NIA solubility was determined in PBS, methanol and water-methanol (50:50) in order to identify a suitable receptor solution for permeation and mass balance studies. An excess of the compound was placed in an Eppendorf® tube containing 0.5 mL of solvent. The tube was shaken for 5 min using a vortex shaker and then placed in an orbital shaker at 32°C for 24 h. The samples were then centrifuged at 13000 rpm at 32°C for 15 min. Supernatant solution was removed and diluted for HPLC analysis.

2.4 NIA content of formulations

The amount of NIA in each formulations was determined by taking known amounts of the formulation in Eppendorf® tubes (n=3). One mL of water:methanol (50:50) solvent mixture was added to the tubes which were shaken for 5 min using a vortex shaker and then placed in an orbital shaker at 32°C for 24 h to extract NIA from the formulations. After 24 h, the tubes were centrifuged at 32°C, 12000 rpm and for 15 min. The supernatant solution was removed, diluted with water:methanol (50:50) and analysed by HPLC.

2.5 Permeation and mass balance studies

Permeation studies of NIA in Franz diffusion cells were conducted at three different doses (50, 20 and 5 µL) in silicone membrane and porcine skin as described in detail previously [11,12]. Freshly prepared PBS (pH 7.3 ± 0.1) was used as the receptor solution. Once the skin/membrane temperature had equilibrated to $32\pm 1^\circ\text{C}$, the formulation was applied using an Eppendorf® Multipette Plus. The donor compartment was occluded using Parafilm® for infinite and pseudo finite doses (20 and 50 µL) and not occluded for the finite dose (5 µL) application. A volume of 200 µL of receptor solution was removed from the receptor compartment at various time intervals up to 24 h, with sample replacement using fresh temperature equilibrated PBS solution. All samples were analysed using HPLC.

At the end of the permeation studies, the skin or membrane surface was washed 5-times for the 20 and 50 µL applications and 3-times for the 5 µL applications with 1 mL of water:methanol

(50:50) followed by swabbing with a cotton bud. For each washing step, the surface was rinsed 5-times with the same washing solution. The samples were placed in separate Eppendorf® tubes which were placed on a vortex mixer for 15 min at room temperature. Skin was removed from the cells and cut into small pieces with scissors and placed in an Eppendorf® tube with 1 mL of water:methanol (50:50). For the 50 µL application skin samples were extracted twice. The first extraction was conducted for 16 h in an orbital shaker at 32°C. The skin samples were then removed from the tubes and excess liquid was blotted using Kimberly Clark® tissue paper. The second skin extraction was conducted by incubating the samples with 1 mL of water:methanol (50:50) for 3 h in an orbital shaker at 32°C. For the 5 and 20 µL applications, the skin and membrane samples were extracted once by incubation with 1 mL of water:methanol (50:50) for 16 h in an orbital shaker at 32°C. All samples were centrifuged at 12000 rpm at 32°C for 15 min. The supernatant solution was then collected, diluted where necessary and analysed using HPLC. Mass balance data were calculated using Equations 1 and 2.

$$T = W + E + P \dots\dots\dots \text{Equation 1}$$

Where T=total recovery, W= recovery from the surface (by washing), E= recovery from the skin or membrane (by extraction) and P= recovery from the receptor compartment (cumulative permeation at 24 h). Total absorption (A) of NIA by the barrier membranes were also calculated by Equation 2 [13].

$$A = E + P \dots\dots\dots \text{Equation 2}$$

2.6 Statistical analysis

Results are presented as mean± standard deviation (SD). Statistical analysis was performed using MS Excel® and SPSS software (IBM SPSS Statistics, Version 22). One way analysis of variance (ANOVA) followed by a Post Hoc Tukey test was conducted for multiple comparison between the groups. A value of p<0.05 was considered as a statistically significant difference.

3. Results and discussion

3.1 Solubility of NIA in water, methanol and PBS

The solubility values of NIA in PBS, methanol and water:methanol (50:50) are shown in Table 2 and confirm PBS as a suitable receptor medium for permeation studies. As the solubility of NIA was 2.6 fold higher in water:methanol (50:50) compared with pure methanol the former was selected for

washing and extraction of NIA in mass balance studies. The water methanol solution (50:50) was also used to extract and assay NIA in all formulations.

Table 2. Solubility of NIA in PBS, methanol and water:methanol at 32°C at 24 h (mean±SD, n=3)

Solvent	Solubility of NIA (mg/mL)
PBS	85.64±17.77
Methanol	175.62±19.91
Water:methanol (50:50)	469.06±10.25

3.2 NIA content in formulations

The amount of NIA in Formulations A and B were determined to be 2.92 and 3.17 %w/w, respectively, which were ~ 97 and 106% of the claimed content; an amount of 3.69 %w/w of NIA was determined for the commercial product (Table 3).

Table 3. Assay results of NIA for all formulations (mean±SD, n=3)

Formulation	Claimed amount (%w/w)	Assay (%w/w)
Commercial control	-	3.69±0.12
Formulation A	3	2.92±0.09
Formulation B	3	3.17±0.03

3.3. Permeation and mass balance results

Permeation studies of NIA from the three formulations were conducted using silicone membrane and porcine skin and with various amounts of formulations, namely infinite, pseudo finite and finite doses. The silicone membrane was selected as a model membrane because of its homogeneity and it also allows insight into the influence of excipients on membrane transport [14,15].

For the 50 μL dose, in both membranes, absolute NIA permeation may be ranked as follows Control > Formulation A > Formulation B (Figures 1a and b). For silicone membrane, NIA permeation from commercial control ($151.0 \pm 34.4 \mu\text{g}/\text{cm}^2$) was significantly higher only from Formulation B ($110.4 \pm 9.2 \mu\text{g}/\text{cm}^2$, $p < 0.05$) and not significantly higher than from Formulation A ($138.2 \pm 13.0 \mu\text{g}/\text{cm}^2$, $p > 0.05$) despite the commercial control containing a significantly more NIA than Formulation A. In porcine skin significantly higher amounts of NIA permeated at 24 h from commercial control ($254.7 \pm 50.9 \mu\text{g}/\text{cm}^2$) compared with Formulations A and B, respectively ($p < 0.05$). For the 20 μL dose, similar quantities of NIA permeated from the three formulations in porcine skin (Figure 1c) ranging from 180.0 – 245.8 $\mu\text{g}/\text{cm}^2$ ($p > 0.05$). For the same dose in silicone membrane (Figure 1d) NIA permeation was significantly different at 24 h between all formulations with values of 163.23 ± 19.1 , 120.2 ± 15.2 and $89.7 \pm 11.3 \mu\text{g}/\text{cm}^2$, respectively, for the control, Formulation B and Formulation A ($p < 0.05$). For the infinite and pseudo-finite doses (50 and 20 μL), permeation of NIA from all formulations was higher in porcine skin (Figures 1a,1c) compared with silicone membrane (Figures 1b and 1d). As expected, a non-linear permeation profile was observed for the finite (5 μL) dose in both membranes (Figures 1e, 1f). Permeation of NIA in porcine skin at 24 h was almost two-fold higher for commercial control ($41.3 \pm 16.5 \mu\text{g}/\text{cm}^2$) compared with Formulations A and B ($p < 0.05$). Similar amounts of NIA permeated for Formulation B and C (21.9 ± 12.6 and $21.1 \pm 9.5 \mu\text{g}/\text{cm}^2$, $p > 0.05$) in porcine skin. No differences in amounts permeated were observed for silicone membrane (77.0 - $89.0 \mu\text{g}/\text{cm}^2$) for the finite dose application ($p > 0.05$). Higher permeation is evident in porcine skin compared with silicone membrane for the 50 and 20 μL doses but the opposite trend is observed for the 5 μL dose. This may reflect the more exaggerated effects of excipients on the barrier function of biological tissue when applied in large doses.

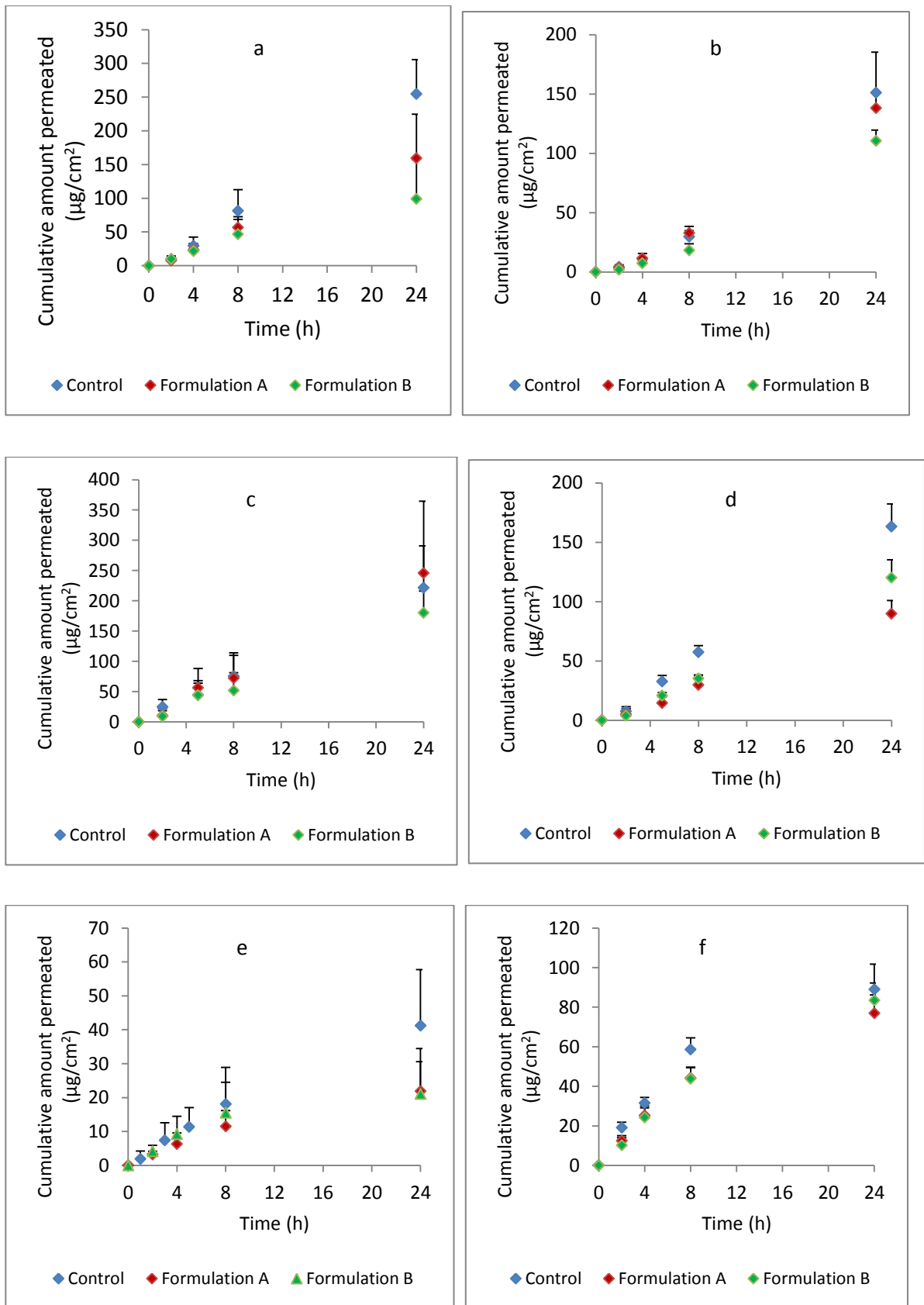


Figure 1. Permeation profiles of NIA from three formulations (a): 50 μL porcine skin (b) 50 μL silicone membrane (c) 20 μL porcine skin (d) 20 μL silicone membrane (e) 5 μL porcine skin and (f) 5 μL silicone membrane (mean \pm SD, n= 5-10)

In porcine skin, for the infinite dose (50 μ L) the percentage permeation ranged from 7-14% and for the finite dose (5 μ L) the corresponding value was 17-31%; for the 20 μ L dose the percentage permeation ranged from 34 – 51%. In silicone membrane, the percentage permeation of NIA at 24 h decreased with increasing dose of formulation; the percentage permeation ranges for infinite, pseudo-finite and finite doses were 8.4 -11, 19 - 27 and 57 -71%, respectively. Mass balance studies were carried out after each permeation study and the results are shown in Table 4.

Table 4. Skin extraction and absorption of NIA for all formulations for 50, 20 and 5 μ L doses in porcine skin and silicone membrane (mean \pm SD, n \geq 5)

Dose applied	Barrier membrane	Formulation	% Extraction	% Absorption
50 μ L	Porcine skin	Control	2.00 \pm 1.04	16.06 \pm 4.47
		Formulation A	2.88 \pm 1.67	15.05 \pm 6.47
		Formulation B	1.52 \pm 0.89	8.16 \pm 4.53
	Silicone membrane	Control	0.33 \pm 0.22	8.92 \pm 0.95
		Formulation A	0.42 \pm 0.14	11.38 \pm 2.09
		Formulation B	0.52 \pm 0.41	8.93 \pm 1.06
20 μ L	Porcine skin	Control	2.10 \pm 0.51	38.54 \pm 8.45
		Formulation A	3.25 \pm 1.85	54.56 \pm 21.81
		Formulation B	2.41 \pm 1.26	36.34 \pm 6.78
	Silicone membrane	Control	1.02 \pm 0.34	27.54 \pm 3.67
		Formulation A	0.81 \pm 0.09	19.83 \pm 3.55
		Formulation B	0.64 \pm 0.12	22.78 \pm 1.50
5 μ L	Porcine skin	Control	10.04 \pm 5.32	41.25 \pm 9.68
		Formulation A	9.63 \pm 6.05	29.02 \pm 12.76
		Formulation B	15.65 \pm 8.10	32.71 \pm 6.87
	Silicone membrane	Control	1.96 \pm 0.67	58.79 \pm 4.92
		Formulation A	1.91 \pm 0.92	73.64 \pm 6.59
		Formulation B	1.50 \pm 0.38	70.86 \pm 2.73

For the three doses, retention or deposition of NIA was generally higher in porcine skin compared with silicone membrane. Comparable amounts of NIA were deposited in skin for all formulations for all doses and total skin absorption values are also similar for all formulations (p>0.05). Previously it has been shown that the SPF containing prototype formulation containing lamellar bilayer structures was able to localise UV filters into the outer SC. [16] This demonstrates that the prototype formulation can effectively deliver ingredients to where they are needed for performance in the skin for optimum efficacy. For the finite dose (5 μ L), deposition in porcine skin was 5 to 10 fold higher than the silicone membrane (Table 4) which may reflect differences in

thickness and/or lipophilicity of the membranes [17]. Total absorption values are also highest for all formulations in silicone membrane for the finite dose studies. Comparatively higher absorption levels ($p < 0.05$) are evident for the 20 μL dose in silicone membrane (20 to 28%) than the 50 μL dose (9 to 11%). For the different dosing conditions, absorption levels in skin may be ranked as follows 20 μl = 5 μl > 50 μl . The skin absorption value of NIA from formulations for the 5 μl dose are comparable to results reported by Franz for *in vitro* finite dose studies of NIA; following application of 4 $\mu\text{g}/\text{cm}^2$ of NIA to human abdominal skin, a total of 28.8% of the active was absorbed [18]. Absorption values for the 50 μl dose conditions are also consistent with previous values for infinite dose permeation studies where 10.9% absorption of NIA was observed for permeation from a 10% (v/v) aqueous ethanol solution [7]. For the 50 μL dose, there was no significant difference in NIA absorption from the commercial control and the barrier-mimetic formulations ($p > 0.05$) in both membranes. Similar findings are evident for the 20 and 5 μL doses in porcine skin ($p > 0.05$). However, NIA absorption was significantly higher for the commercial control compared with Formulation A ($p < 0.05$) for the 20 μl dose in silicone membrane.

For all doses of all formulations applied to silicone membrane, the total recovery values of NIA were within $100 \pm 15\%$. These values lie within the acceptable limit of recovery (85-115%) established by the Scientific Committee on Consumer Safety for dermal absorption studies [19]. In porcine skin the recovery values for the 50 μl dose lay within the SCCP guidelines for all formulations. However for the 20 and 5 μl doses, amounts recovered for the skin studies lay outside the recommended recovery limits and may reflect the difficulties in achieving total recovery of the formulations in the donor compartment for smaller doses. To overcome this concern, different methods are being developed to identify possible chemical transition states that may affect NIA throughout the permeation process. This would explain the absence of NIA in lower dosage permeation studies and allow the differentiation between a NIA protonated / deprotonated state. This will be the focus of a future study.

Conclusions

Despite the higher content of NIA in the control formulation there were no differences in amounts of NIA absorbed between this formulation and the skin barrier-mimetic formulations. Absolute permeation of NIA in porcine skin was significantly higher for the control formulation compared with both test formulations (Formulation A and B) at infinite (50 μL) and finite (5 μL) applied dose-levels. This may reflect the differences in formulation composition between control

and Formulations A and B, along with the higher initial level of NIA present in the control formulation. For the inert silicone membrane, for the 50 μL dose, permeation of NIA from the control formulation and Formulation A were similar and for the 5 μL dose permeation of NIA from all formulations was comparable. For the pseudo-finite dose (20 μL) the amounts of NIA appearing in the receptor solution were comparable for porcine skin. For the 50 and 20 μL doses higher absolute permeation was observed in porcine skin compared with silicone but the opposite was the case for the 5 μL dose. This may reflect an artefactual influence of excipients on permeation when large doses of formulations are applied. The results indicate (i) the critical importance of dosing for evaluation of topical formulations (ii) the ability of the skin barrier-mimetic formulations to deposit similar amounts of NIA in the skin compared with a higher strength control formulation.

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