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Topical delivery of niacinamide: influence of neat solvents

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ABSTRACT

Niacinamide (NIA) has been widely used in cosmetic and personal care formulations for several skin conditions. Permeation of topical NIA has been confirmed in a number of studies under infinite dose conditions. However, there is limited information in the literature regarding permeation of NIA following application of topical formulations in amounts that reflect the real-life use of such products by consumers. The aim of the present work was therefore to investigate skin delivery of NIA from single solvent systems in porcine skin under finite dose conditions. A secondary aim was to probe the processes underlying the previously reported low recovery of NIA following *in vitro* permeation and mass balance studies. The solubility and stability of NIA in various single solvent systems was examined. The solvents investigated included Transcutol® P (TC), propylene glycol (PG), 1-2 hexanediol (HEX), 1-2 pentanediol (1-2P), 1-5 pentanediol (1-5P), 1-3 butanediol (1-3B), glycerol (GLY) and dimethyl isosorbide (DMI). Skin permeation and deposition of the molecule was investigated in full thickness porcine skin *in vitro* finite dose Franz-type diffusion experiments followed by mass balance studies. Stability of NIA for 72 h in the solvents was confirmed. The solubility of NIA in the solvents ranged from 82.9 ± 0.8 to 311.9 ± 4.5 mg/mL. TC delivered the highest percentage permeation of NIA at 24 h, 32.6 ± 12.1 % of the applied dose. Low total recovery of NIA after mass balance studies was observed for some vehicles, with values ranging from 55.2 ± 12.8 % to 106.3 ± 2.3 %. This reflected the formation of a number of NIA degradation by-products in the receptor phase during the permeation studies. Identification of other vehicles for synergistic enhancement of NIA skin delivery will be the subject of future work.

Key words: *In vitro*, porcine skin, permeation, finite dose, niacinamide

1. Introduction

Niacinamide (NIA) also termed nicotinamide is a water-soluble vitamin that has been used in a range of topical formulations (Cosmetic Ingredient Review Expert Panel, 2005). The molecule is naturally found in human cells as a precursor of the co-dehydrogenases NAD and NADP and participates in several biological oxidation – reduction reactions. NIA has been reported to demonstrate various effects *in vitro*, namely (i) increased cellular DNA synthesis (ii) repair of UV-induced DNA damage in human melanocytes (iii) promotion of synthesis of ceramides, free fatty acids and cholesterol in cultured human keratinocytes (iv) inhibition of melanosome transfer from melanocytes to keratinocytes in co-cultured cells and (v) decreased IL-8 production and inhibition of *P. acnes* induced inflammatory responses of human keratinocytes (Tanno et al., 2000; Hakozaki et al., 2002; Grange et al., 2009; Thompson et al., 2014).

The actions of NIA on human skin health have also been investigated in a number of *in vivo* studies. Crowther et al. (2008) used confocal Raman spectroscopy to measure the stratum corneum (SC) thickness and water content of the forearm of 14 human subjects after application of various emollient formulations twice daily for 2 weeks, one of which contained NIA. The NIA-containing formulation significantly increased SC thickness and hydration compared with other treatments and the control (untreated site). A later *in vivo* study was conducted by Mohammed et al. (2013) for 28 days. Formulations containing 5 % NIA as well as control formulations were applied twice daily on the forearms of 20 volunteers. At the end of the study areas treated with niacinamide showed decreased trans-epidermal water loss (TEWL) and increased SC thickness, larger and more mature corneocytes as well as decreased inflammatory activity.

In a more recent clinical study, Nisbet et al. (2019) investigated the changes in facial redness of 59 female participants with winter xerosis-associated skin erythema. The study included two test treatment groups with volunteers that applied two different NIA formulations twice daily for 28 days. The researchers found a statistically significant reduction in facial redness for both groups after 15 days compared with a baseline. They also reported that after 29 days of treatment the level of efficacy was greater for subjects with severe erythema compared with those with moderate facial redness. Topical application of NIA has also been reported to have a role in reduction of sebum production (Draelos et al.,

2006). Recently, Caetano et al. (2019) conducted an *in vivo* study to examine the effects of a topical formulation containing NIA in female subjects. A significant reduction in both papule and pustule count and appearance after 8 weeks was reported for the group that used the NIA-containing product compared with the control. Finally, the ability of NIA to reduce the appearance of hyper-pigmentation has also been reported *in vivo*. Navarrete-Solis et al. (2011) examined the depigmentation effect of topical NIA on 27 female patients' facial skin with melasma over an 8-week period. The patients applied a NIA formulation on one side of their face and a hydroquinone preparation on the other side. Hydroquinone is an established ingredient for skin whitening (Jimbow et al., 1974) and treatment with NIA resulted in similar whitening effects on the hyper-pigmented areas of the skin as hydroquinone.

Although NIA is a hydrophilic compound with a $\log P_{(o/w)}$ value of -0.35 (Haque et al., 2017a), the ability of the molecule to penetrate the skin after topical application has been confirmed by several *in vitro* studies using human or porcine skin (Cosmetic Ingredient Review Expert Panel, 2005; Mohammed et al., 2014; Haque et al., 2017a; Zhang et al., 2019a). However, there is only limited information with regards to skin permeation of NIA using finite doses of formulations (Haque et al., 2017a; Zhang et al., 2019a). Where such studies have been conducted, low recovery of NIA was also observed following mass balance studies (Haque et al., 2017a; Zhang et al., 2019a). Although this has recently been investigated using pseudo-finite and infinite dose models (Sil et al., 2018), evaluation of the potential degradation process has not been examined under finite dose conditions. The aims of the present work were therefore (i) to investigate the skin delivery of NIA *in vitro* in porcine skin from a range of single solvents under finite dose conditions and (ii) to utilise LC-MS in order to gain insight into the processes underlying the low recovery of NIA in these studies.

2. Materials and methods

2.1. Materials

NIA, 1-2 propanediol (PG), 1-2 pentanediol (1-2P), 1-5 pentanediol (1-5P), 1-2 hexanediol (HEX), 1-3 butanediol (1-3B), dimethyl isosorbide (DMI), dipropylene glycol (DiPG) and glycerol (GLY) were supplied by Sigma Aldrich (Dorset, UK). Methanol and water hyper grade for LC-MS, LiChrosolv® were also obtained from Sigma-Aldrich (Dorset, UK).

Transcutol® P (TC) was a kind donation from Gattefossé (St. Priest, France). High Performance Liquid Chromatography (HPLC) grade water and methanol were purchased from Fisher Scientific (Leicestershire, UK). Phosphate-buffered saline (PBS) tablets were purchased from Oxoid Limited (Cheshire, UK). Full thickness porcine ear skin was obtained from a local abattoir from healthy animals farmed for food chain purposes.

2.2. Methods

2.2.1. HPLC analysis and method validation

The analysis of NIA was performed using an HPLC system of 1260 series (Agilent Technologies, Santa Clara, CA, USA), equipped with a Luna® reverse phase column, 250 x 4.60 mm, 5 µm, Phenyl-Hexyl (Phenomenex, Macclesfield, UK) and a universal HPLC guard column (Phenomenex, Macclesfield, UK) packed with a SecurityGuard™ cartridge (Phenomenex, Macclesfield, UK). The analytical method as described previously (Haque et al., 2017a) was optimised and validated for the parameters of linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) (International Council for Harmonisation, 2005). The mobile phase consisted of methanol:water (20:80). The UV detector was set to 263 nm, the flow rate to 1 mL/min and the column temperature to 30^{o} C. The injection volume was 10 µL. A calibration curve ranging from 0.5 to 200 µg/mL was constructed. The validation parameters of accuracy, precision, inter-day variability and intra-day repeatability were determined as described previously (Iliopoulos et al., 2019).

2.2.2. Dynamic vapour sorption studies

A dynamic vapour sorption (DVS) apparatus, TGA Q5000 SA (TA Instruments, New Castle, DE, USA) was used to study the mass differences due to evaporation or hydration of the examined formulations. Metallised quartz pans connected to a microbalance accurate to 0.1 % of the measured weight were used to evaluate the mass variations. Temperature and relative humidity (RH) were controlled throughout the experiment at 32 ± 0.5 °C and 50 ± 2 % RH, respectively. 5 µL of formulation were placed in the sample pan for 24 h, i.e. solutions of NIA (3 % w/w) in PG, 1-2P, 1-5P, 1-3B, GLY, DMI, DiPG and TC. Data were recorded using Universal Analysis 2000 v. 4.5A (TA Instruments, New Castle, DE, USA)

2.2.3. Solubility parameter, solubility and stability studies

The solubility parameters (δ) of both the actives and solvents were estimated by the Van-Krevelen and Hoftyzer method using Molecular Modelling Pro® software (Version 7.0.8, Norgwyn Montgomery Software Inc., North Wales, PA, USA, 2016). The solubility parameters are defined as the sum of all the cohesive forces of a material. The Van Krevelen and Hoftyzer approach has been used for rational selection of excipients in pharmaceutical formulations and good correlation between theoretical predictions and experimental findings has been reported (Shah and Agrawal, 2013). Saturation solubility determinations were conducted as reported previously: excess NIA was added to each solvent mixture in Eppendorf® tubes in triplicate. Tubes were placed in a shaking incubator (Orbital Mini shaker, VWR International Limited, Leicestershire, UK) for 48 h at 32 ± 1° C and were subsequently centrifuged for 15 min at 10,000 rpm and 32° C in an Eppendorf 5415R centrifuge (Eppendorf, Hamburg, Germany). The supernatant liquid was diluted as necessary to lie within the analytical range of the HPLC method (Iliopoulos et al., 2019). Stability of NIA in the solvents was investigated for 72 h (3 d) at 32 ± 1° C. Solutions of NIA 3 % (w/w) were prepared in triplicate and placed in an Eppendorf ® tube. The sample was sealed with Parafilm® (Bemis Inc., U.S.A.) and placed in a shaking incubator, as for solubility studies. Samples were collected at 0, 24, 48, and 72 h. All samples were diluted and analysed by HPLC.

2.2.4. Finite dose permeation and mass balance studies

NIA solutions (3 % w/w) were prepared, as formulations of this concentration have previously shown effective permeation through skin (Haque et al., 2017a). The permeation studies were conducted using vertical glass Franz-type diffusion cells and full thickness porcine ear skin according to procedures reported previously (OECD, 2004a, b; Haque et al., 2017b; Iliopoulos et al., 2019). Freshly prepared PBS (pH 7.3 ± 0.2) was used as the receptor solution. Finite doses of 5 µL of the test solution were applied to the donor compartment which was not occluded. A volume of 200 µL of receptor solution was collected at 0, 15 min, 30 min, 45 min, 1 h, 2 h, 5 h, 8 h, 10 h, 12 h and 24 h, and an equal volume of PBS solution was added to the receptor compartment. All samples were analysed using HPLC. The number of replicate experiments was $n \geq 4$. After the 24 h permeation study, a mass balance study

was also conducted as described previously (Haque et al., 2017a). The skin surface was washed and the skin membranes were extracted with 1 mL of water:methanol (50:50) to quantify the amount of NIA on the surface and inside the skin respectively. The skin samples were centrifuged for 1 min at 13,000 rpm at 32^{o} C in an Eppendorf 5415R centrifuge (Eppendorf, Hamburg, Germany) and subsequently they were extracted by incubation in an orbital shaker (Orbital Mini shaker, VWR International Limited, Leicestershire, UK) at 32^{o} C overnight. Subsequently, all samples were centrifuged at 13,000 rpm at 32^{o} C for 20 min, the supernatant solution was collected and analysed using the HPLC analytical method.

2.2.5. Identification of NIA degradation by-products following *in vitro* skin permeation studies

The analysis of NIA and NIA by-products was conducted as described by Sil et al. (2018). Briefly, at the end of the permeation study, the receptor solution was collected and freeze-dried using a Thermo Scientific Heto Power dry LL1500 manifold freeze dryer equipped with a RC 6 chemistry-HYBRID rotary high vacuum pump (Vacuubrand®, Brackley, UK) and condenser temperature set at -113°C. Potential NIA by-products were extracted from the dry residue with water:methanol (50:50) and the samples were analysed by LC-MS. Subsequently, semi-preparative LC was performed to isolate the unknown peak with a retention time 4.6 min (Figure S1), which was further investigated with accurate mass analysis. The LC-MS analysis of the samples was conducted using an Agilent Infinity II 1260 (Agilent Technologies, Cheshire, UK) system equipped with an Agilent G7129A vial sampler, G7111B quaternary pump and G7114A variable wavelength detector coupled to a single quadrupole mass spectrometer Agilent Infinity Lab LC-MSD XT. The parameters for the mass spectrometry analysis were the same as those reported by Sil et al. (2018), and the conditions were the same as for the HPLC method. For the semi-preparative LC, a sample volume of 100 µL was injected. High-resolution mass spectra were carried out using either a Kratos MS89MS with Kratos DS90 software or a Jeol AX505W with Jeol complement data system. Samples were ionised electronically (EI), with an accelerating voltage higher than 6 kV or by low resolution fast atom bombardment (FAB) in a thioglycerol matrix.

2.2.6. Data analysis

The data was analysed using Microsoft® Excel 2013 software (Microsoft Corporation, Redmond, Washington, USA) and GraphPad Prism Statistics software (version 8.3.0, San Diego, CA, USA, 2019). Results are presented as the mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test. For parametric data, the independent-samples t-test as well as one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test were used to analyse two groups and ≥ 3 groups respectively. For non-normally distributed data or where variances were not equal, the Kruskal-Wallis and Mann-Whitney U Tests and independent sample t-test were used, respectively. Statistical significance was assumed when the p value was less than 0.05.

3. Results and discussion

3.1. HPLC method validation

A correlation coefficient value (r^2) greater than 0.99 was obtained, thereby indicating an acceptable fit to the regression line (Shabir, 2003). The retention time of NIA was 5.2 ± 0.1 min. The accuracy was 100.2 ± 0.5 %. The % RSD values for inter-day and the intra-day precision were 0.29 ± 0.19 % and 0.26 ± 0.19 %, respectively. The LOD value was 0.15 µg/mL and the LOQ was 0.47 µg/mL.

3.2. Solubility and stability of NIA in candidate vehicle

The saturation solubility values of NIA in the solvents investigated are shown in Fig. 1. The calculated solubility parameters of vehicles are also shown. The solubility parameter of NIA was calculated as 13.9 $((cal/cm^3)^{1/2})$. NIA solubility in the solvents ranged from 82.9 ± 0.8 to 311.9 ± 4.5 mg/mL (Figure 1). Solubility of NIA was found to be higher in the solvents with solubility parameter values closer to that of NIA. This observation is consistent with other reports in the literature (Mohammed et al., 2014; Zhang et al., 2019a). NIA demonstrated stability in all solvents, with recovery values > 85 % after 72 h, as shown in Fig. S2.

3.3. Dynamic vapour sorption studies

Fig. 2 shows the DVS results for all NIA 3 % (w/w) single solvent solutions over 24 h at 32 ± 0.5 °C and 50 ± 2 % RH. The most volatile solvent was found to be TC and only 6.3 % of the initial dose was recovered at 24 h. For PG, 52.9 % of the applieS2d mass was recovered at 24 h. 26.7 % and 21.7 % of the applied DMI and 1-2P respectively evaporated. Only 5% of the applied DiPG evaporated during the experiment. For all solvents, an initial increase of weight was found because of their hygroscopic nature. No evaporation was evident for the GLY and 1-5P formulations. These findings are consistent with previous reports on the evaporation of neat GLY, DMI, PG and TC (Haque et al., 2017b; Zhang et al., 2019b). To our knowledge DVS has not been used previously to characterise the behaviour of NIA solutions under controlled conditions of temperature and humidity. DVS results for the neat solvents suggest that both PG and TC will evaporate to a much greater extent compared with the other solvents after 24 h, as shown in the Figure 2.

3.4. Permeation studies using neat solvents

Finite dose permeation studies of NIA were performed using GLY, DiPG, PG, HEX, 1-2P, 1-5P, 1-3B, DMI and TC. All permeation studies were conducted for 24 h with samples (200 µL) taken at different time points (t = 0, 15 min, 30 min, 1h, 2h, 5h, 8h, 10h, 12h and 24h). A dose of 5 µL of each formulation was applied on the surface of the skin. Permeation of NIA was evident for all solvents, as shown in Figure 3. For NIA to exert its actions as described in the introduction above, it must reach the viable layers of the skin. The cumulative permeation of an active *in vitro* is an indicator of the effectiveness of the respective formulations, in terms of promoting its delivery to the skin under real conditions *in vivo*, as demonstrated in previous studies (Mateus et al., 2014; Mohammed et al., 2014).

The highest percentage permeation of NIA after 24 h was from TC, comprising 32.6 ± 12.1 % of the applied dose. This value was not significantly different from that for PG (19.2 ± 16.3%, $p > 0.05$), but significantly higher than for all other solutions investigated ($p < 0.05$). Even though the mechanism by which TC interacts with the skin is not known, its potential to act as a penetration enhancer has been reported for a number of compounds (Lane, 2013). Puglia and Bonina (2008) investigated the effect of several commonly used enhancers on the permeation of atenolol in human skin *in vitro.* The authors prepared five emulsions, each containing a different penetration enhancer and reported that the TC

containing formulations resulted in the highest drug absorption. This was attributed to the ability of TC to penetrate SC and increase solubilisation of active inside the skin. More recently, Haque et al. (2017b) used gas chromatography to measure percutaneous absorption of solvents *in vitro* and found that TC permeated through human skin in significantly higher quantities compared with other solvents following finite dosing. In addition, the volatile nature of the solvent TC has been reported previously (Haque et al., 2017b) and the evaporation of NIA-containing TC formulations was confirmed experimentally in the present study (Figure 2). Depletion of the solvent from the skin surface can increase the thermodynamic activity of the active in the vehicle, and it is likely to result in enhancement of percutaneous absorption. This hypothesis has been examined by Oliveira et al. (2012) who conducted *in vitro* finite dose permeation studies in human skin using different vehicles with and without ethanol. Formulations containing ethanol were reported to result in increased penetration of the active compound, which was attributed to partial evaporation of the formulations leaving a highly concentrated residue on the skin surface. In the present study, we found that 93.7 % of the initial mass of the NIA in TC solution had evaporated after 24h (Figure 2). The evaporation of the formulations as examined by DVS and shown in Figure 2 is not expected to be the same as when the formulations are applied to the skin in a Franz cell. Specifically, unlike the conditions in the DVS chamber, where a constant flow of nitrogen is present to maintain the desired relative humidity, the donor chamber of a Franz cell may inhibit the air movement, thereby decreasing the evaporation rate of the volatile compounds (Reifenrath et al., 1991). Additionally, permeation of the solvents and the active inside the skin can lead to a decrease of the residue on the upper skin layer and this cannot be estimated by the DVS studies. However, despite these limitations, it is clear that TC is a volatile solvent and notable amounts of this vehicle will evaporate from the skin surface over 24 h. Even though TC does not evaporate as rapidly as ethanol (Oliveira et al., 2012), the dynamic nature of the TC preparation is likely to promote similar enhancing effects on delivery of NIA over a 24 h period.

PG is a hydrophilic solvent that has been used in topical formulations as a solvent for many actives (Lane, 2013; Haque et al., 2017b; Haque et al., 2018; Paz-Alvarez et al., 2018; Hossain et al., 2019; Iliopoulos et al., 2019; Zhang et al., 2019b). In the present work, 5 µL doses of 3 % (w/w) NIA in PG were applied to porcine skin and the percentage permeation after 24 h was 19.2 %. These data are consistent with a recent report from our group, where 19.5 % of the dose of NIA permeated across pig skin after a finite dose application of 5 % (w/v) NIA in PG (Zhang et al., 2019a). The mechanism of action of PG has not been fully

understood, however, it is believed that it penetrates the SC and increases the solubility of the active in the skin (Williams and Barry, 2012; Lane, 2013). Permeation of PG through human skin has been confirmed in a number of *in vitro* studies. Trottet et al. (2004) conducted Franz-type experiments using gel formulations containing PG and found that the percentage permeation of PG was approximately 30% when finite doses of the formulations were applied. In a later study, Haque et al. (2017b) applied similar amounts of neat PG to human skin *in vitro* and reported that 13 % of the applied solvent had permeated at 24 h. In an *in vivo* study, Mohammed et al. (2014) monitored the penetration of NIA and various solvents across the SC using confocal Raman spectroscopy. These authors noted that penetration of NIA was correlated with the depth of penetration of the PG, indicating the ability of PG to "carry" the active as it diffuses across the skin. In the present work, PG was the only solvent that promoted NIA permeation to the same extent as TC ($p > 0.05$).

DiPG is a hydrophilic oligomer of PG, that has been widely used in skin formulations (Cosmetic Ingredient Review Expert Panel, 1985), but its effect on the skin barrier has not been studied in detail. Fasano et al. (2011) investigated the permeation of both PG and DiPG across human abdominal skin using infinite dose *in vitro* studies under occlusion. The researchers also measured the skin impedance before and after the experiments. DiPG was reported to have no effect on the impedance values after 24 hours of skin exposure, while PG caused a significant decrease in impedance. DiPG was reported to have a 2.5 fold lower steady state permeation flux than PG under these conditions. Lower skin permeation of DiPG than PG was also reported by Haque et al. (2017b) following finite dose permeation studies in human skin. The percentage permeation of DiPG was reported to be 2.6 % at 24 h and 8 % at 48 hours, and it was significantly lower than permeation of PG. In the present work, both solvents delivered similar amounts of NIA across porcine skin ($p > 0.05$); however, NIA permeation from DiPG was significantly lower than from TC ($p < 0.05$).

DMI is commonly used as a solubiliser in topical formulations. The exact mechanism of its interaction with the skin remains unknown and contrasting reports of its potential to act as a penetration enhancer can be found in the literature. Generally, it is believed that this solvent can promote topical delivery of hydrophilic compounds by increasing the polarity of the SC and therefore promoting drug partitioning in this layer. In an *in vivo* study, confocal Raman spectroscopy was used to monitor the penetration of both DMI and NIA across the SC. As for PG, a linear correlation between signal intensity of DMI and the signal intensity of

NIA was found (Mohammed et al., 2014). However, in a later study, DMI had no enhancing effect on the penetration of hexamidine diisethionate nor its dihydrochloride salt across pig skin *in vitro* (Parisi et al., 2016). In the present work, single solvent systems of DMI resulted in cumulative permeation of NIA of 9.8 ± 2.7 μg/cm^2, corresponding to 6.1 ± 2.1% of the applied NIA. These values were significantly lower than TC ($p < 0.05$), but comparable with all other solvents ($p > 0.05$).

The solvent 1-2P is a short chain 1, 2-glycol that is used mainly as a skin conditioning and viscosity increasing agent in topical products (Johnson et al., 2012). There is only limited information in the literature regarding its action on the skin and its ability to act as a penetration enhancer has not been established. Duracher et al. (2009) examined the influence of 1-2P on the permeation of caffeine across porcine skin *in vitro* following finite dose application under occlusion. The researchers compared the efficacy of vehicles consisting of water and either 1-2P or PG and reported that the 1-2P formulations delivered higher amounts of caffeine through the skin. Additionally, the authors prepared formulations of 1-2P:water at various ratios and examined their efficacy. The solutions of 1-2P:water (5:95) resulted in twice as much penetration of caffeine compared with 1-2P:water (2.5:97.5), indicating that the glycol enhanced permeation of caffeine in a dose dependent manner.

HEX is a solvent commonly used as a preservative in personal care products because of its antibacterial properties (Yogiara et al., 2015). Given its chemical structure, it might be assumed that, as for other 1,2 glycols, HEX is likely to penetrate the skin and increase permeant solubility in the SC. Lee et al. (2011) conducted a 24 h Franz-diffusion study to measure the penetration of various neat 1,2-alkanediols in porcine skin and reported that HEX permeated porcine skin at a similar rate as 1-2P. In the present work, permeation of NIA was comparable from both HEX and 1-2P solutions (9.4 ± 1.0 and 9.4 ± 1.6 μg/cm^2 respectively, $p > 0.05$).

The solvent GLY is widely used in topical products and has been found to improve hydration of the skin in a number of clinical studies (Batt and Fairhurst, 1986; Batt et al., 1988; Pedersen and Jemec, 1999). Even though it has been used mainly as a humectant rather than a penetration enhancer, it is believed that increased hydration of the SC can increase mobility of the lipids and affect penetration of actives (Zhang et al., 2005). Brinkmann and Müller-Goymann (2005) used various techniques, such as differential scanning calorimetry

(DSC), wide and small angle X-ray-diffraction (WAXD and SAXD) to investigate the effects of GLY on human skin. These authors reported that GLY interacts with SC lipids in a similar manner as PG, by integrating between the polar head groups and disordering the skin lipids. We recently investigated the effect of GLY on the permeation of the hydrophilic compound 3-O-ethyl-l-ascorbic acid in porcine skin after finite dosing and found that it resulted in similar permeation of the active as for PG (Iliopoulos et al., 2019). In this present work, cumulative permeation of NIA from GLY was 7.3 ± 2.4 μg/cm^2 after 24h. This value was lower than the cumulative permeation of NIA from TC (50.8 ± 19.0, $p < 0.05$). However, GLY delivered comparable amounts of NIA as the other solvents, PG, DMI, 1-2P, 1-3B, HEX and 1-5P ($p > 0.05$). The solvent 1-3B, butylene glycol, has also been used as a solvent in many topical formulations (Cosmetic Ingredient Review Expert Panel, 1985). Haque et al. (2017b) examined the penetration of this solvent in human skin; 10.3 % of the applied solvent permeated the skin after 48 h. In the present work, neat 1-3B resulted in cumulative permeation of NIA of 6.1 ± 0.4 μg/cm^2, which corresponds to 4.2 ± 0.2 % of the applied NIA.

1-5P is a short chain alkanediol and its use as a solvent in topical formulations is relatively recent. It has been used as an antimicrobial ingredient but its potential to enhance topical delivery has not been established (Sundberg and Faergemann, 2008). In a number of studies in the literature 1-5P has been reported to enhance penetration of certain lipophilic actives in human skin *in vitro*. Evenbratt and Faergemann (2009) evaluated the effects of 1-5P and PG on percutaneous delivery of terbinafine. These workers prepared 1% terbinafine gel formulations containing various concentrations of either 1-5P or PG and a control formulation containing no alkanediol. The researchers proposed that both solvents increased permeation of terbinafine compared with the control. They also found that formulations containing 5 % 1-5P enhanced topical delivery of the active more effectively than those containing 5 % PG. In later study, Faergemann et al. (2005) investigated the ability of a commercial cream containing hexylene glycol and a similar formulation containing 1-5P, to promote skin delivery of mometasone furoate. Studies were conducted in human skin using continuous flow diffusion studies *in vitro*. Similar permeation of mometasone furoate from both preparations was observed, suggesting that the two solvents had similar enhancing effects on percutaneous absorption of the active. With regards to hydrophilic active ingredients, however, no penetration of an ethyl derivative of vitamin-C in porcine skin *in vitro* was found when 1-5P was used as a neat solvent (Iliopoulos et al., 2019). The contrasting effects of the solvent on the permeation of different actives might be attributed to

their different physicochemical properties. It is not known if 1-5P is able to permeate across the SC, however, given its hydrophilic nature ($logP_{(o/w)}$ = -0.49 at 25 °C (MSDS, 2019)), it is possible that it may not diffuse through the skin, but instead remain in the upper layers of the SC. In this work, 1-5P resulted in significantly lower permeation of NIA through pig skin compared to TC ($p < 0.05$). 1-5P delivered similar amounts of NIA (6.1 ± 0.4 μg/cm^2) compared with the other solvents ($p > 0.05$).

The total recovery of NIA after mass balance studies was outside the acceptable range of 90 - 110 % for the vehicles TC (55.2 ± 12.8 %), PG (58.9 ± 12.7 %), DMI (69.5 ± 15.3 %), 1-2P (72.3 ± 10.5 %), DiPG (74.9 ± 11.9 %) and HEX (79.4 ± 18.5 %), as shown in Table I (OECD, 2004a, b). The total recovery values observed for 1-5P and GLY, 106.3 and 103.4 % respectively, were significantly higher than those for TC and PG ($p < 0.05$). The percentages of NIA recovered for DMI, DiPG, 1-2P and HEX were comparable to values for PG and TC ($p > 0.05$). Low recovery of NIA following finite dose porcine skin permeation studies has previously been reported in the literature. Haque et al. (2017a) conducted finite and pseudo-finite permeation studies in porcine skin using various formulations containing NIA 3 % (w/w). The researchers reported that total recovery of NIA after 24 h was lower than 85 % for all formulations, indicating partial degradation of NIA. More recently, Zhang et al. (2019a) conducted finite dose porcine skin Franz diffusion and mass balance studies using solutions of NIA 5 % (w/v) and reported low total recovery values for a number of solvents, including PG, TC and DMI.

3.5. Identification of NIA degradation by-products following *in vitro* skin permeation studies

As noted, a number of pseudo-finite or finite dose porcine skin permeation studies of NIA have been reported in the literature for several formulations. The total NIA recovered in mass balance studies fell below the acceptable limits required by OECD guidelines for the majority of the solvents used (OECD, 2004a, b; Mohammed et al., 2014; Haque et al., 2017a; Zhang et al., 2019a). The findings of the present work are consistent with the previous reports. Recently, Sil et al. (2018) investigated this phenomenon using infinite and pseudo-finite dose *in vitro* studies in porcine skin with subsequent mass spectrometry analysis and suggested that the low recovery reflected chemical derivatisation of the molecule during the permeation experiment. The researchers proposed that a reduction pathway of NIA occurred,

leading to the formation of several by-products i.e. piperidine-3-carboxamide, 1,4,5,6-tetrahydropyridine-3-carboxamide and 1,4-dihydropyridine-3-carboxamide.

In this work, finite doses (5 μL/cm^2) of 3 % NIA (w/w) in either TC or PG were applied to porcine skin to investigate the low recovery of NIA. Following LC-MS analysis of the receptor solution and subsequent characterisation of the mass spectrometry data, the compound 1,4,5,6-tetrahydropyridine-3-carboxamide was identified (*m/z* 127 for M+H, Figure 4). The *m/z* 173 indicates the presence of the ion adduct of this molecule for M+H+2Na. The *m/z* 149 indicates the presence of the M+Na ion of the same compound. A value of *m/z* 171 correlates with the presence of 1,4-dihydropyridine-3-carboxamide for M+H+2Na. These findings confirm the occurrence of the proposed NIA reduction pathway as described by Sil et al. (2018), where it was proposed that NIA transformed to 1,4-dihydropyridine-3-carboxamide, subsequently converting to 1,4,5,6-tetrahydropyridine-3-carboxamide and finally to piperidine-3-carboxamide. In the present work, the piperidine-3-carboxamide compound was not found in the LC-MS spectrum after the finite dose experiments.

The unknown compound was separated from the sample by semi-preparative LC. Subsequently, accurate mass analysis was conducted and the presence of 1,4,5,6-tetrahydropyridine-3-carboxamide was confirmed. Specifically, the accurate mass analysis for NIA and 1,4,5,6-tetrahydropyridine-3-carboxamide is as follows: *m/z* 123 (100%, [M+H]+): Found $[M+H]^+$ 123.0555, $C_6H_6N_2O$ requires 123.0558 and *m/z* 125 (100%, $[M-H]^-$): Found $[M-H]^-$ 125.0342, $C_6H_{10}N_2O$ requires 125.0715.

The results of this work identified a number of NIA degradation by-products following the finite dose skin penetration studies. These findings confirm the occurrence of a reduction pathway of NIA as proposed by Sil et al. (2018). However, the final stage of the pathway, namely the formation of the degradant, piperidine-3-carboxamide, was not apparent (Figure 5). This could be attributed to the lower doses of NIA employed in the present study compared with the infinite or pseudo-finite dose studies performed in the earlier work. The formation of these NIA by-products likely explains the low total recoveries observed following mass balance studies.

4. Conclusions

Topical formulations of NIA have demonstrated a number of beneficial effects on skin health and barrier function. This study examined the effect of a number of solvents on NIA topical delivery in porcine skin *in vitro*. Of the solvents studied, TC delivered the highest amount of NIA through the skin compared with other solvents with cumulative NIA permeation of 50.8 ± 19.0 μg/cm^2, corresponding to 32.6 ± 12.1 % of the applied dose. The low recovery of NIA after finite dose permeation studies was probed and provided a number of insights into the chemical transformation of NIA during penetration. The identification of a number of NIA by-products should allow a greater understanding of the fate of NIA in the skin. This knowledge will give additional insights into the delivery of NIA inside the skin, and will enable a more precise assessment of the efficacy of cosmetic or pharmaceutical products on NIA percutaneous delivery. Future studies will focus on additional techniques, such as diffusion NMR to separate and quantify the various by-products observed at the end of the permeation studies. Subsequently, additional experiments using well-established techniques that can assess the skin barrier function, such as trans-epidermal water loss (TEWL) and confocal Raman spectroscopy (CRS) will be conducted for investigation of potential pharmacological role of these by-products on the skin. Moreover, knowledge of the effects of commonly used vehicles on NIA topical delivery will enable synergistic combinations of solvents to be selected for future formulation development. Finally, the efficacy of complex solvent systems will be investigated and comparisons with commercially available products will be investigated.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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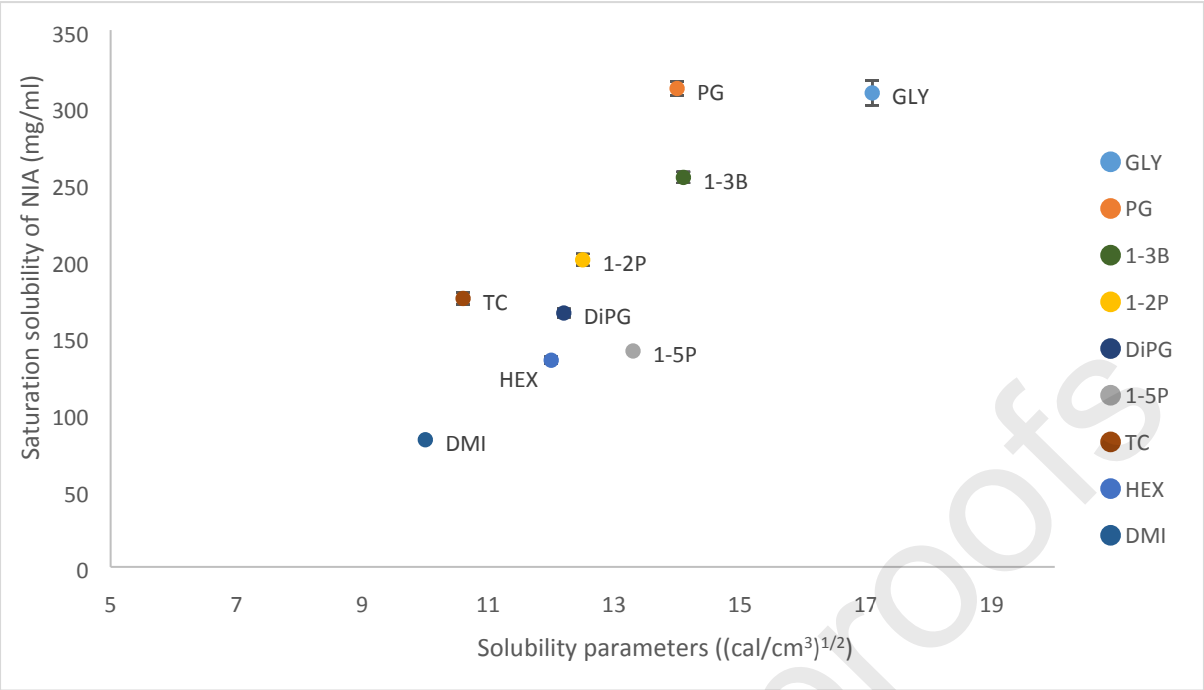

Figure 1 – Solubility parameter values plotted against saturation solubility of niacinamide in selected solvents at 32 ± 1 °C (n = 3, mean ± SD).

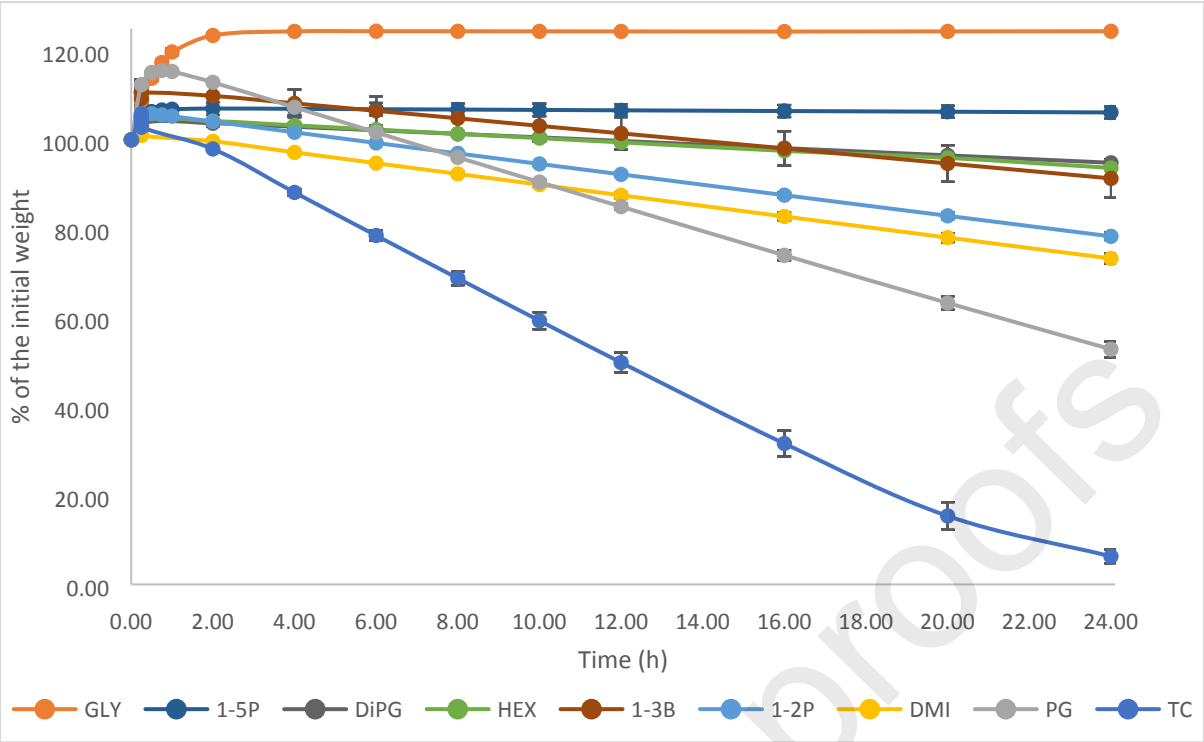

Figure 2. Percentage of weight loss from NIA 3% (w/w) solutions in various solvents over 24 h at 32 ± 0.5 °C and 50 ± 2 % RH (n ≥ 3, mean ± SD).

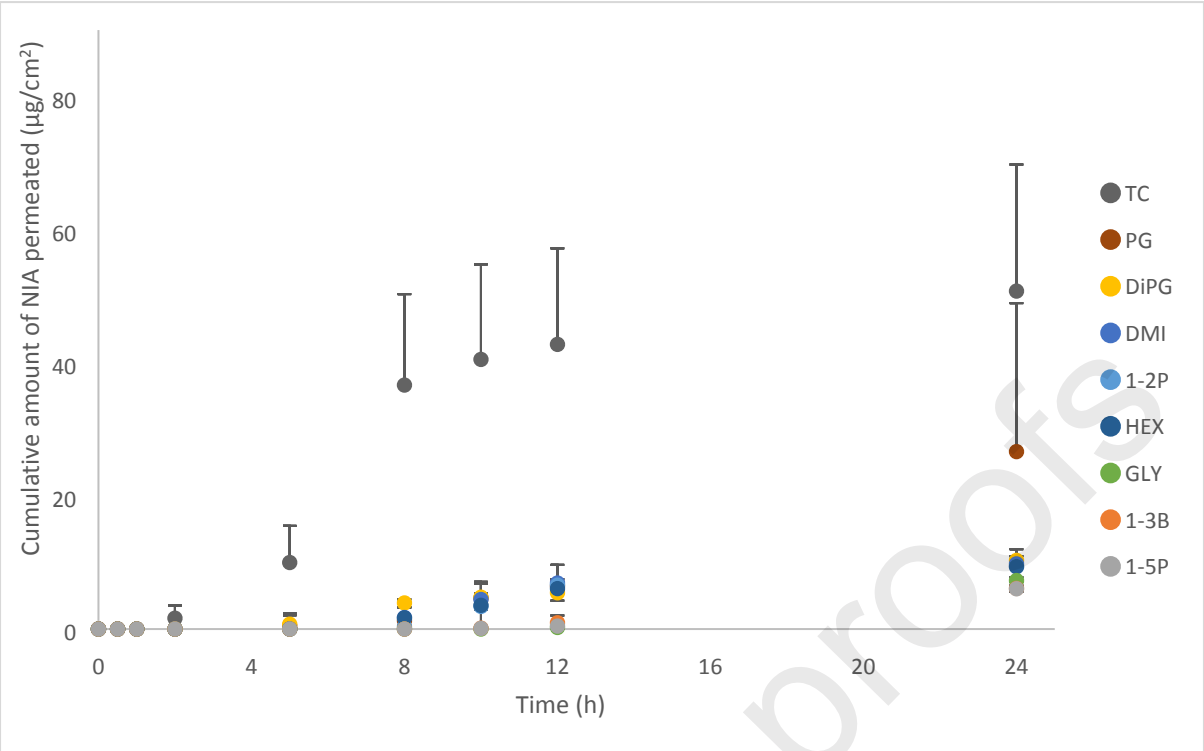

Figure 3 – Cumulative amount of niacinamide that permeated over time for neat solvents in porcine skin – Finite dose (n ≥ 4; mean ± SD, *p < 0.05).

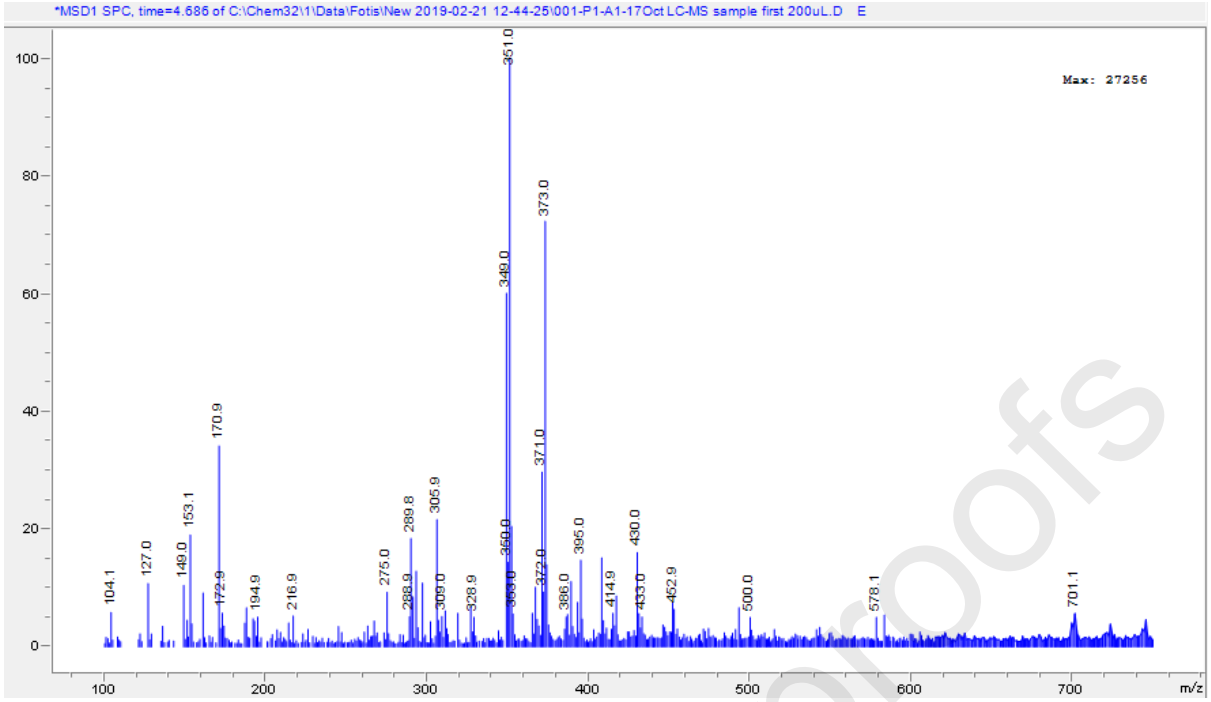

Figure 4 - Niacinamide (NIA) by-products mass spectrometry (MS) spectrum

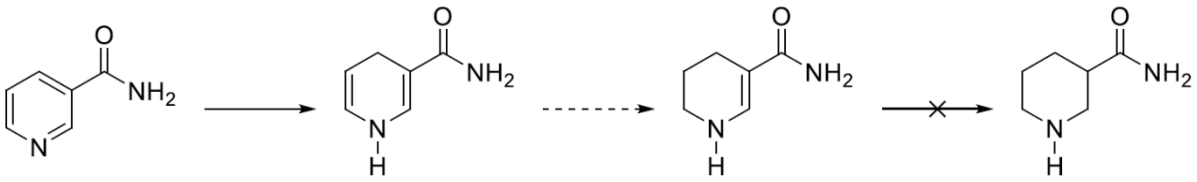

Figure 5 – Niacinamide (NIA) reduction pathway for the formation of its by-products after finite dosing

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Table I – Percentage (%) permeation, skin extraction, recovery from skin surface and total recovery of niacinamide for neat solvents (mean ± SD, n ≥ 4).

Solvent	Percentage (%) of applied dose			
	% extraction	% permeation	% washed from the surface	% total recovery
GLY	6.0 ± 1.1	4.0 ± 1.3	93.5 ± 8.6	103.4 ± 7.7
PG	16.5 ± 4.2	19.2 ± 16.3	23.2 ± 2.4	58.9 ± 12.7
DiPG	5.6 ± 1.1	6.5 ± 0.9	62.8 ± 12.7	74.9 ± 11.9
1-2P	19.9 ± 2.7	7.9 ± 1.1	44.5 ± 11.9	72.3 ± 10.5
HEX	20.1 ± 6.5	8.2 ± 1.5	51.1 ± 25.5	79.4 ± 18.5
TC	5.1 ± 1.2	32.6 ± 12.1	17.5 ± 0.8	55.2 ± 12.8
1-3B	6.2 ± 0.5	4.2 ± 0.2	86.4 ± 7.5	96.8 ± 8.0
1-5P	5.0 ± 1.0	4.5 ± 0.6	101.8 ± 2.1	106.3 ± 2.3
DMI	4.6 ± 0.6	6.1 ± 2.1	58.8 ± 16.9	69.5 ± 13.3