



Research Article

Topically Applied Active Ingredients Establish an Equilibrium Dependent on Their Physico-Chemical Properties and a Skin Layer Specific

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Abstract

Topical administration presents an alternative route for targeted active ingredient delivery. Thus, an increasing demand for reliable ex vivo data to predict the outcome of in vivo skin penetration abilities of active ingredients arises. It is important to understand the impact of donor differences for percutaneous skin penetration studies and the influences of the physico-chemical properties of actives.

The skin penetration abilities of two active ingredients with different lipophilicity and similar molecular mass were determined. Franz diffusion cell penetration studies with a finite and infinite topical dosing and subsequent skin separation of the Stratum Corneum (SC), epidermis, and dermis layer were accomplished. For lipophilic active ingredients, the epidermis and dermis are the rate-limiting skin layer to enable targeted penetration, whereas for hydrophilic actives the penetration rate is limited by the SC. In this study, we found that the tissue capacity and the ability of the different skin layers to hold a maximum amount of active ingredient varies from donor to donor, but shows an equilibrium for each active compound for a specific donor. This novel result, to our knowledge, has not been described in literature thus far and would help optimize dosing strategies and formulation development as well as to better understand the pharmacokinetics of topically applied active ingredients.

Keywords: Franz diffusion cell; Inter-Donor equilibrium; Inter-Donor differences; Tissue saturation; Transdermal; Skin penetration; Caffeine diffusion

Introduction

The skin with a surface area of approximately 2 m² is the biggest organ in adult humans and provides a physical barrier to the environment, limits water loss, and enables protection against microorganisms, and toxic agents [1, 2]. Nevertheless, the skin provides an essential route for the targeted delivery of active ingredients inside the human body and a fundamental

morphological understanding is required [3]. A key challenge for percutaneous penetration studies is to detect and quantify the targeted delivery of active ingredients to evaluate a dermal and transdermal uptake [3,4].

The skin with its multicellular structure provides a barrier function of the body and is divided into an epidermal and dermal layer. The epidermis is subdivided into the viable epidermis (E) and the outermost layer, the stratum corneum (SC), which acts with its enucleated corneocytes as the main barrier for the penetration of compounds [3]. The E, below this cornified layer, comprises of keratinocytes which are precursors to corneocytes of the SC.

In addition to keratinocytes, this layer contains melanocytes, Langerhans cells, and Merkel cells and is, therefore, region for drug binding and metabolic response [5-7]. The dermis (D), is lies under the E in the skin and includes fibroblasts, which secrete a dense irregular collagen network that forms a connective tissue with its extracellular matrix structure [8]. Besides this connective tissue, the D contains sensory nerve endings, blood vessels, and immune cell systems and is therefore conducive to the targeted delivery of active ingredients [9-12].

For the development of topical administered biologically active ingredients, the determination of a local drug concentration in different skin layers is essential to achieve targeted delivery. To ascertain this ex vivo percutaneous penetration experiments can be carried out, with the most common method being the Franz Diffusion Cell (FDC) setup [13]. Here porcine ear skin is a widely used surrogate model for ex vivo human skin due to its similar penetration characteristics, along with its morphology [14,15]. To reduce experimental variability, 500 μm thick split-skin is obtained and separated into SC, E and D after the penetration experiment to determine donor to donor differences [3]. The efficacy of topical applied active ingredients is most often limited due to its poor skin penetration ability rather than the potency of the molecule itself [16].

Topical applied actives can permeate, penetrate, and diffuse into the skin via several penetration routes: [1] the intercellular lipid route between the cells, [2] the transcellular route crossing through the cells, and [3] the follicular route through the hair follicle shaft [17]. Compounds with a lipophilic character are known for a higher ability to pass the SC via the intercellular route, whereas hydrophilic compounds are more likely to use the transcellular route [18]. Due to differences in a preferred primary penetration route, a diverse set of physicochemical active ingredient characteristics was chosen for this study. Caffeine is recommended by the Organization for Economic Co-operation and Development (OECD) as a hydrophilic model compound due to its extensively studied ex vivo penetration abilities [19,20]. With an identical molecular mass and different lipophilic properties, LIP1 provides with its reliable determination in different skin layers the second model compound for a comprehensive penetration study (Table 1). Thereby Propylene Glycol (PG) as a simplified formulation, is an often used vehicle to topically apply active ingredients and shows synergistic effects in combination with oleic acid (OA) to enable skin penetration studies [18]. The amount of the topically applied formulation varies from a finite dose ($\sim 10\mu\text{L}/\text{cm}^2$) to an infinite dose ($>100\mu\text{L}/\text{cm}^2$) [21,22]. Infinite dosing enables to evaluate of the absolute active concentration within the different skin layers to ensure efficacy and exposure to the target tissue.

	Caffeine	LIP1
Chemical composition	$\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$	$\text{C}_9\text{H}_{10}\text{N}_2\text{O}_3$
Molecular mass [g/mol]	194.19	194.19
Log P	-0.1 (exp.)	0.6 (exp.)
Melting point [°C]	236 (exp.)	179 (exp.)
Solubility in H₂O [25°C]	21.6 mg/mL (exp.)	0.75 mg/mL (exp.)
Solubility in PGOA [25°C]	7.2 mg/mL (exp.)	52.0 mg/mL (exp.)
CAS Number	58-08-2	65609-28-1

Table 1: Physico-chemical properties of caffeine and LIP1.

Suitable ex vivo models are indispensable to identify targeted skin penetration into the different tissue compartments and need to show reliable and reproducible bioequivalent correlation to in vivo [22]. Porcine penetration studies give insides into the distribution, accumulation, and saturation capacity of active ingredients in different skin layers and ensure penetration-depth profiling [23]. Thereby the interpretation of intra-donor variations combined with inter-donor differences is important to understand the penetration of actives on human skin [24,25]. For an early-stage selection criterion, reliable skin penetration data and influences of the physicochemical properties of actives on their penetration ability are important for the development of new chemical entities and formulations.

In this study, we describe the influences of hydrophilic and lipophilic active ingredients with identical molecular mass on their skin penetration behavior. The main focus is to interpret intra- and inter-donor variations on the ability of active ingredient localization and to identify the maximum tissue capacity of different skin layers for these actives via an infinite formulation dosing.

Material & Methods

Reagents

Caffeine, LIP1 (synthesized internally), water (Milli-Q®) and acetonitrile from Merck KGaA (Darmstadt, Germany). Phosphate Buffered Saline (PBS) (w/ magnesium w/ calcium), Propylene

Glycol (PG), and Oleic acid (OA) from Sigma Aldrich by Merck KGaA (St. Louis, USA). Cyanoacrylate super glue from UHU GmbH & Co. KG (Bühl, Germany).

Formulations

Caffeine PGOA

PGOA with 5% OA (w/w) were prepared with a concentration of 0.7% caffeine (w/w).

LIP1 PGOA

PGOA with 5% OA (w/w) were prepared with a concentration of 0.7% LIP1 (w/w).

Materials

2- and 5-mL tubes from Eppendorf (Hamburg, Germany). Omnifix-F 3 mL syringes from B. Braun (Melsungen, Germany). Millex[®] syringe filter units (0.22 μ m PES) and Chromolith[®] Performance HPLC column (RP-18e 100 x 4.6 mm) from Merck KGaA (Darmstadt, Germany). HPLC screw micro-vials from VWR (Darmstadt, Germany). Cotton wipes from dm-drogerie markt GmbH + Co. KG (Karlsruhe, Germany). Tape strips (TesaFilm[®] crystal clear 4129) from Beiersdorf (Hamburg, Germany).

Porcine Skin

Pig ears (German domestic pigs, 6-month-old) obtained freshly from a local slaughterhouse (Brensbach, Germany) were washed with water, dried, and stored at +4°C for a maximum of 72 h. The skin from the back of the ear was dermatomed using a dermatome from Humeca BV (Borne, Netherlands) at an average thickness of 500 μ m, measured using an electrical digital micrometer (0-25 mm/0.001 mm) from Vogel Germany GmbH & Co. KG (Kevelaer, Germany). Six skin discs (\varnothing 25 mm) were obtained from each ear.

Franz Diffusion Cell (FDC)

The skin was placed onto the FDC (orifice \varnothing 9 mm, diffusion area 0.64 cm²) from Logan Instruments Corp. (Somerset, USA) dermal side down on the receptor chamber. The receptor chamber filled with PBS was constantly stirred and temperature controlled (32 \pm 1°C). Before the donor chamber was placed onto the skin, the transepidermal water loss (TEWL) was measured (n=5) with an open chamber TEWL device (Dermalab series) from Cortex Technology (Hadsund, Denmark). After an equilibration time of 30 min, 10 μ L/cm² (finite) or 786 μ L/cm² (infinite) of the PGOA formulation containing 0.7% of the active ingredient, was topically applied.

Skin Separation

To determine the amount of the active ingredient in the different skin layers the Stratum Corneum (SC), viable Epidermis

(E), and Dermis (D) were separated individually. After 4 h the penetration experiment was terminated, the Receptor Fluid (RF) was mixed taking an aliquote of 2 mL using a filter unit. The donor chamber was washed using 2 mL PBS and the formulation residues were removed from the skin using a cotton wipe which was collected in 2 mL PBS, both being defined as skin wash (SW).

SC Removal

The SC was removed by using tape stripes and cyanoacrylate glue (n=5). After an incubation of 2 min, the strips were removed against the grain of hair growth and collected in 7 mL PBS.

E/D Removal

For the E and D separation, the application site was punched out of the skin discs. Placed dermal side down for 90 s on a 60°C heating plate from IKA[®] (Staufen, Germany), and the E was carefully peeled off using forceps. The E and D layers were weighed and collected in a 2 mL tube each. The remaining skin discs (Lateral) without the application site, were collected in 2 mL tubes. To the E, D, and Lateral tissue 1 mL PBS and a 5 mm stainless steel ball were added and the tubes were shaken for 10 min at 30 Hz (2x) using a TisseLyser II from Qiagen (Hilden, Germany) and the balls were removed afterward. For the E and D tissue, 1 mL and for the Lateral tissue 0.5 mL PBS was added additionally. All samples were incubated for 20 h at 60 rpm using a tube roller from Stuart equipment (Staffordshire, UK) and filtered afterward.

High-Performance Liquid Chromatography (HPLC)

The caffeine and LIP1 concentrations of the samples were determined using an ELITE LaChrom HPLC system from VWR-Hitachi (Darmstadt, Germany). The analytical determination was performed using a Chromolith[®] Performance column with a flow rate of 2.0 mL/min and an isocratic method of Acetonitrile/water for caffeine (10:90) and LIP1 (20:80). The quantitative amount of caffeine and LIP1 has detected at a wavelength of 272 nm and 285 nm with a detection unit (DAD I-2450) from VWR-Hitachi (Darmstadt, Germany) and a column temperature of 30°C. The samples were mixed and transferred into micro vials prior to the analysis with an injection volume of 60 μ L (n=4). To ensure chemical stability the samples were analyzed latest 7 d after the penetration experiment. The specificity of each HPLC run was controlled using an internal standard solution as a blank injection. The caffeine and LIP1 detection and quantification limits were confirmed by a six-point calibration curve for caffeine (0.5-250 μ g/mL) and LIP1 (0.125-62.5 μ g/mL) before and after the sample detection (n=3). A graphical linear regression was confirmed with a R² > 0.99 and within an accepted accuracy and precision criteria <2% in all cases.

Statistical Analysis

Calculations were performed using Microsoft® Excel® Office 365 (Dublin, Ireland) and all data are shown as mean ± SD. The statistical analysis was performed using GraphPad Prism 7.03 (San Diego, USA), with statistical analysis being one-way ANOVA (Bonferroni method) and a p-value of <0.05 considered as statistically significant.

Results & Discussion

To conclude from ex vivo percutaneous penetration studies to in vivo safety and efficacy the Scientific Committee on Consumer Safety and the OECD recommend to use of various donors for penetration experiments [14,20]. Therefore, the comparability of FDC penetration studies using one, two, or three different donors and the determination of the barrier function as well as the diffusivity of active ingredients within the different skin layers was determined. The intra- and inter-donor variability and the maximum active amount in different skin layers were tested in a comprehensive study, using two active ingredients with identical molecular mass and different lipophilic properties. For topical application, a vehicle containing caffeine (hydrophilic) or LIP1 (lipophilic) needs to enable epidermal permeability, and a combination of PG and OA is often used [26,27]. This topically applied formulation diffuses over time and 4 h penetration experiments are commonly used to assure the maximum penetration [28,29]. For a concentration-depth profiling approach and to determine the amount of active ingredient within the different layers, the SC was removed via cyanoacrylate stripes, and the E was separated from the D via heat separation [30,31]. The amount of accumulated active ingredient within the different skin strata, as well as the flux through the skin into the RF which represents systemic uptake, was defined. For topical delivery systems and tissue bioavailability, the accumulation in the skin with minimal penetration is desired, whereas for systemic delivery the opposite is preferred. [23] Therefore the inter-donor variations for skin integrity and penetration ability are known, but the saturation capacity of the different layers is still unclear [21,32].

The barrier function of the Different Skin Layers

The distribution of caffeine and LIP1 within the different skin layers for a finite percutaneous penetration experiment was determined for three different donors (Figure 1). The cumulative mean distribution of the active ingredients was compared and for caffeine, 53.31 % penetrated inside the RF, and 11.42 %, 1.02

%, and 4.54 % were determined within the SC, E, and D layers, respectively (Figure 1A). For LIP1 33.41 % penetrated inside the RF and 31.42 %, 2.58 %, and 6.13 % active was found in the SC, E, and D layers, respectively (Figure 1B). This penetration flux into the RF and the concentration of active ingredients in the different tissue layers is equivalent to other lipophilic actives and caffeine penetration data, with the same tissue ratio [29,33]. Therefore, compared to their hydrophilic counterparts, lipophilic active ingredients appear to be distributed at higher concentrations in the SC, which is described to be the main contributing factor to the barrier function of the skin (34).

For the three individual caffeine penetration experiments Donor 3 shows a higher amount of active ingredient within the SC, E, and D than Donor 2 and this in turn higher than Donor 1, whereas the amount of penetrated caffeine within the RF remained the same (Figure 1B). LIP1 shows a higher overall tissue concentration compared to hydrophilic caffeine with Donor 3 > Donor 2 > Donor 1 comparing the different skin layers (Figure 1C). The concentration of penetrated LIP1 inside the RF is opposite to the overall tissue concentration with Donor 3 containing the lowest and Donor 1 the highest concentrations respectively. Differences between penetration studies using multiple donors and variability for an RF penetration of different compounds have been described previously in the literature [35]. This study shows that an overall higher concentration of lipophilic active ingredients within the tissue leads to tissue saturation and the maximum tissue capacity for this active is reached for these specific skin layers. The SC is described as a more lipophilic barrier, whereas the viable epidermis and D are considered primarily hydrophilic phases [34,36]. This would lead to the conclusion, that the barrier function of the skin is not limited by the barrier function of the SC, depending on the lipophilicity of the active ingredient, the other skin layers also influence the penetration kinetics [37]. The nature of the active ingredient itself dictates its primary penetration route although multiple routes are used simultaneously [18]. Of the factors contributing to the nature of an ingredient, lipophilicity, and molecular size are known to affect the penetration and its affinity to the skin surface as well as its compatibility with the intercellular SC lipids [18]. For hydrophilic active ingredients, the partitioning out of the vehicle into the lipophilic SC and for lipophilic actives the partitioning out of the SC into the E is rate limiting [28]. Therefore a steady-state flux and a tissue saturation of active ingredients can be reached via an infinite topical application amount to determine the maximum capacity of the skin [38].

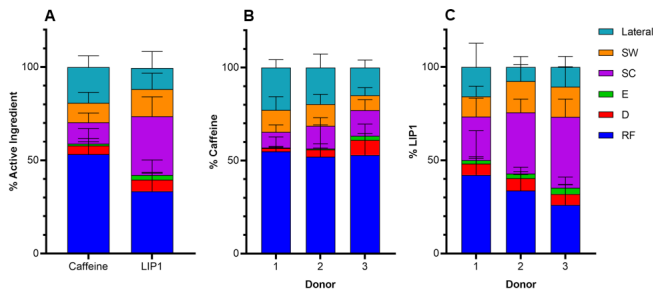


Figure 1: The cumulative mean distribution (A) of caffeine and LIP1 and the mean distribution of caffeine (B) and LIP1 (C) within the different skin layers of porcine skin after a FDC penetration experiment was determined. A finite dose of 10 $\mu\text{L}/\text{cm}^2$ of a PGOA formulation containing 0.7 % active ingredient was topically applied for 4 h on 500 μm thick porcine split-skin at 32°C. Each bar shows the percentage of determined active ingredient in the different layers (Lateral, SW, SC, E, D and RF) after the skin penetration experiment. The cumulative mean distribution within three different donors (n=18) and the mean distribution within one donor (n=6) for three different donors was determined. Values represent mean \pm SD and a total recovery of active ingredient > 70 % of the application amount, compared using one-way ANOVA.

Saturation of the Different Skin Layers

The cumulative mean concentration of caffeine and LIP1 normalized to skin layer mass for a finite (10 $\mu\text{L}/\text{cm}^2$) and infinite (786 $\mu\text{L}/\text{cm}^2$) percutaneous penetration experiment were determined (Figure 2A). For an infinite applied formulation dosing the SC layer shows a significantly higher concentration of caffeine and LIP1, with the highest concentration observed for the infinite caffeine formulation (Figure 2B). Whereas LIP1 shows a significantly higher concentration for the finite and infinite applied amount compared to the finite caffeine experiment. Within the E and D LIP1 shows a higher active concentration per skin layer mass compared to the caffeine concentration for the finite applied volume, with no significant difference (Figure 2 C/D). Whereas the infinite applied caffeine formulation shows compared to the other experiments a higher concentration for the E (11 - 13-fold) and D (13 - 27-fold) with a significant difference. This could indicate that the SC comprises for both active ingredients a higher

maximum saturation capacity per skin layer mass compared to the E and D. But in fact, for each active ingredient the E shows a comparable maximum saturation capacity as in the case for the D if a steady-state flux is reached. For LIP1 the infinite applied formulation compared to the finite experiment shows a doubling of the concentration of the molecule in the E and a similar concentration in the D. However, neither of these differences are significant for infinite vs finite dosing. Chen et al. describe a comparable increased penetration magnitude for hydrophilic and lipophilic actives by using an infinite formulation amount [39]. Infinite applied dosing does not only lead to a steep concentration gradient with direct action on skin penetration and deposition [36]. It also affects the hydration of the SC which influences the active skin distribution [39,40] and is linked to the degree of formulation evaporation, since the higher the formulation loading, the lower the evaporation that occurs [41]. The partitioning from the formulation into the SC is a limiting step for skin penetration and depends on the physicochemical properties of the active itself, as well as on the hydration state of the SC [36,39]. A higher hydration condition of the SC leads to an increased amount of delivered active, followed by the partitioning into the less lipophilic viable epidermis and deeper skin layers [39]. Additionally, infinite dosing leads to an increased hydrostatic pressure [42] and a mobile disorder and change of SC fluidity [40], which are linked to increased active ingredient penetration. Infinite topical dosing was reported to reach within the SC an active ingredient saturation 1 h after application [43]. Penetration from topical applied formulation is time-dependent and 4-hour penetration experiments are commonly used to represent the maxima of penetration kinetics [28,29]. Here a reservoir and maximum capacity of active ingredients within the SC and its drainage into the E and D via penetration was noticed [29,43]. Nevertheless, an E and D saturation of active ingredients and a maximum capacity of the tissue was to our knowledge not reported before. Since a similar LIP1 concentration within the E and D layer for a finite and infinite application dosing was determined, this study indicates a possible maximum tissue saturation capacity for lipophilic active ingredients. For hydrophilic caffeine, finite and infinite dosing shows significant differences within the tissue, but due to similar concentrations in the E and D for infinite dosing, there is valid evidence that a maximum tissue saturation was reached.

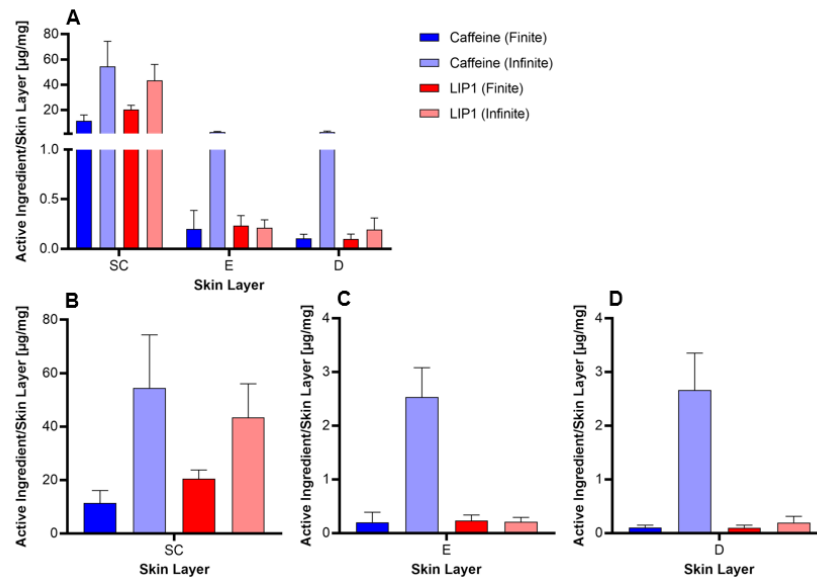


Figure 2: The cumulative mean concentration (A) of caffeine (Blue) and LIP1 (Red) and the mean concentration of caffeine and LIP1 within the SC (B), E (C) and D (D) per skin layer mass of porcine skin after a FDC penetration experiment was determined. A finite dose (darker) of 10 µL/cm² and an infinite dose (lighter) of 786 µL/cm² of a PGOA formulation containing 0.7 % active ingredient was topically applied for 4 h on 500 µm thick porcine split-skin at 32°C. Each bar shows the concentration of active ingredient per skin layer mass (SC, E and D) after the skin penetration experiment. The cumulative mean concentration within three different donors (n=18) and the mean concentration within one donor (n=6) for three different donors was determined. Values represent mean ± SD and a total recovery of active ingredient > 70 % of the application amount, compared using one-way ANOVA.

Intra-donor equilibrium and inter-donor differences

The concentration of caffeine and LIP1 per skin layer mass within the different skin layers for a finite percutaneous penetration experiment was determined for three different donors (Figure 3). The cumulative mean concentration of caffeine per skin layer mass is lower for all skin layers compared to the LIP1 concentration and a significantly higher concentration of each active within the SC compared to the E and D was determined (Figure 3A). The concentration of caffeine per skin layer mass was compared and shows no significant differences between Donor 1 vs Donor 2 vs Donor 3, but a higher concentration of caffeine in all different layers for Donor 3 > Donor 2 > Donor 1 was observed (Figure 3B). Similarly, no significant differences were noticed for LIP1 with the same donor trend and tendency for all skin layers except

for the D of Donor 3. All individuals show an intra- and inter-donor variability for all skin layers for both active ingredients. Southwell et al reported intra- and inter-individual differences in the permeability of human skin and showed a higher inter-donor than intra-donor variation [24]. On the contrary, Meidan et al. reported a smaller skin barrier function between inter-individuals compared to intra-individual [25]. Nevertheless, it is commonly agreed that there exist intra- and inter-donor differences for percutaneous penetration studies. Even so, these differences were found to be non-significant in our penetration experiments. In this study, equilibria between the different skin layers of an individual were identified. These equilibria were found to be specific to individuals with variations between donors. Investigations of these intra-donor equilibria and their inter-donor variations have not been undertaken before, rendering this study novel. (Figure 4).

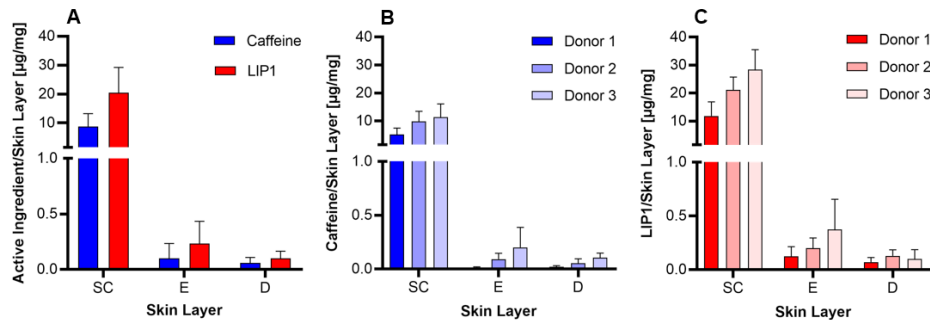


Figure 3: The cumulative mean concentration (A) of caffeine (Blue) and LIP1 (Red) and the mean concentration of caffeine (B) and LIP1 (C) per skin layer mass of porcine skin after a FDC penetration experiment was determined. A finite dose of 10 $\mu\text{L}/\text{cm}^2$ of a PGOA formulation containing 0.7 % active ingredient was topically applied for 4 h on 500 μm thick porcine split-skin at 32°C. Each bar shows the concentration of active ingredient per skin layer mass (SC, E and D) after the skin penetration experiment. The cumulative mean concentration within three different donors (n=18) and the mean concentration within one donor (n=6) for three different donors was determined. Values represent mean \pm SD and a total recovery of active ingredient > 70 % of the application amount, compared using one-way ANOVA.

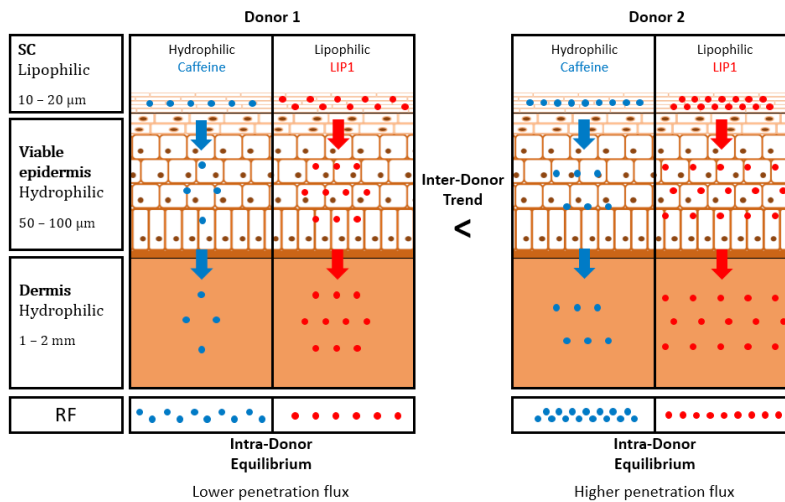


Figure 4: Schematic illustration for a percutaneous penetration study with a where an intra-donor equilibrium with inter-donor differences is reached. Donor 2 shows a 1.5-fold higher saturation of tissue and a maximum active ingredient capacity within the different skin layers compared to Donor 1.

Depending on the individual donor tissue capacity and the ability of the different skin layers to hold a maximum concentration of an active ingredient, varies from donor to donor, but shows an intra-donor equilibrium for each molecule. Therefore, the skin penetration ability for the different skin layers is dependent on the physicochemical properties of the active ingredient and the tissue layers, where each donor shows a unique intra-donor active ingredient capacity and equilibrium. In line with published penetration data, the highest average inter-individual variability, among the different skin layers was exhibited also in this study within the SC layer [44]. Comparing donor differences in general, a sex-based difference between females and males can be neglected, since no significant difference between sexes was observed for dermal penetration studies [45]. Additionally, it was reported, that domestic pigs show compared to humans a lower and less intra- and inter-donor variation [21], with the same magnitude compared to the penetration data shown in this study [46]. This reinforces the validity of this study and that porcine penetration studies demonstrate a reliable ex vivo surrogate to understand

the penetration abilities of active ingredients to predict in vivo penetration in human skin.

Conclusion

In this study, we identified influences of the hydrophilicity and lipophilicity of active ingredients with an identical molecular mass on their skin penetration behavior and defined donor differences. Skin enables an intra-donor equilibrium with inter-donor differences specific for active ingredient characteristics, depending on their physicochemical properties. Each donor shows a maximum tissue capacity for a specific active, which leads to a skin layer saturation, and a novel intra-donor equilibrium is defined. For lipophilic active ingredients, the E and D curb their entry into the tissue whereas, for hydrophilic actives, the SC is the rate-limiting parameter to enable skin penetration. This deeper understanding of how physicochemical properties of ingredients influence their skin penetration ability, and the equilibrium they form in each skin layer is indispensable to the development of NCE and the safety evaluation of topically applied active ingredients.

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Statement of Ethics

This study protocol was reviewed and approved by the administrative office of citizens and regulatory agency, Department of Veterinary Service and Consumer protection, Darmstadt, Germany; approval number [DE06411001721].

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Author Contributions

Conception and design of experiments: Markus Lubda, Inga Pelzer, Andrew Salazar, and Jörg von Hagen; **Acquisition and analysis of data:** Markus Lubda and Inga Pelzer; **Interpretation of data:** Markus Lubda, Inga Pelzer, Jörg von Hagen; **Drafting the work:** Markus Lubda and Inga Pelzer; **Revising the work:** Markus Lubda, Andrew Salazar, and Jörg von Hagen.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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