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In vivo permeation of 2-phenoxyethanol in human skin

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Abstract

A number of baby wipe formulations contain 2-phenoxyethanol (PE) as a preservative and cetylpyridinium chloride (CPC) as a surfactant with antimicrobial activity. Previously, we reported the skin absorption of PE in porcine skin and human skin *in vitro.* In the present work, the permeation of PE from preparations with CPC and without CPC was investigated in human skin *in vivo*. The studies were conducted using Confocal Raman Spectroscopy (CRS) and tape stripping (TS) methods. The CRS studies showed that the area under the curve (AUC) of PE for the formulation with and without CPC were not significantly different ($p > 0.05$). The TS data indicated no significant difference in the amounts of PE recovered from tapes 1-6 for the preparation with and without CPC ($p > 0.05$). When comparing the *in vitro* and *in vivo* data, a correlation was observed between the cumulative amount of PE permeated through human skin *in vitro* at 24 h and the AUC as measured by CRS ($r^2 = 0.97$). In addition, the cumulative amount of PE permeated through human skin *in vitro* at 24 h was found to correlate with the amount of PE recovered from tape 1 to 6 *in vivo* ($r^2 = 0.95$). Both CRS and TS techniques demonstrated limitations in assessing the distribution of PE and CPC in the skin *in vivo*, primarily attributed to the Raman signal intensities of compounds under investigation and the variability in the amount of SC collected by TS. Despite the limitations of CRS and TS, the results from the present study add further insights to the *in vitro* permeation data. Additionally, the findings of the present study encourage the further development and application of CRS for non-invasive evaluation of topical skin formulations *in vivo*.

Keywords
Baby wipes; Confocal Raman Spectroscopy (CRS); Human skin; Infants; Preservative; Skin absorption; Tape stripping (TS)

1. Introduction

In vitro skin permeation is recognized as a well-established method to (i) assess the skin absorption of chemicals for safety evaluation and (ii) to evaluate the percutaneous penetration of drugs from topical and transdermal formulations (Organization for Economic

Cooperation Development, 2004). However, *in vitro* experiments cannot accurately represent in-use conditions. The main advantage of *in vivo* methods for assessing skin penetration and permeation is that the measurements are conducted in a physiologically and metabolically intact system, that accurately mimics in-use conditions (Organisation for Economic Cooperation and Development, 2019; Bielfeldt et al., 2022). More importantly, *in vivo* skin permeation studies in human subjects can provide the most relevant data for assessing the safety and efficacy of the compounds of interest.

Historically, animal models were used to assess dermal absorption of chemicals *in vivo* rather than human subjects, because of ethical considerations (Organization for Economic Cooperation Development, 2004; Organisation for Economic Cooperation and Development, 2019). In the 1980's the technique of tape stripping (TS) emerged as a new approach for investigating skin penetration across the SC in humans (Dupuis et al., 1984; Rougier et al., 1986). To perform TS, the test drug formulation is applied to the skin site, usually the volar forearm. After a specified time period, the SC layers of the application site are removed sequentially by using adhesive tapes. The tape disc is applied to the skin and pressed with uniform pressure to allow adhesion of the SC layer to the tape. The tape is then removed with a constant velocity of removal and the amount of active extracted for quantitative analysis of the active of interest. The limitations of the TS approach have been noted by a number of researchers as well as the US FDA (Bashir et al., 2001; Jacobi et al., 2003; Löffler et al., 2004; Lademann et al., 2009).

More recently, Confocal Raman spectroscopy (CRS) has emerged as a novel analytical technique to monitor skin penetration of various chemicals. It was first proposed for investigating and understanding the structure and composition of the skin (Caspers et al., 2000; Caspers et al., 2001; Egawa et al., 2007). This method is completely non-invasive and is capable of performing real time concentration profiling of actives across the SC *in vivo*. The detection of compounds is based on the Raman spectra acquired by the instrument at different focal depths within the SC. Two wavelength regions are used in this technique, namely the high wavenumber (HWN) region (2500–4000 cm^{-1}) and the fingerprint (FP) region (400–1800 cm^{-1}). The Raman intensities attributed to the compounds under investigation are refined by subtracting the signals from endogenous skin

components. Several studies involving the use of CRS to evaluate *in vivo* permeation of drugs have been published, including retinol (Pudney et al., 2007), ibuprofen (Mateus et al., 2013; Iliopoulos et al., 2023), salicylic acid (Mateus et al., 2014), niacinamide (Mohammed et al., 2014; Zhang et al., 2021), and diclofenac sodium (Iliopoulos et al., 2022). The most recent advances of the CRS technique enable actual quantitative measurements of the actives delivered to the skin (Patel et al., 2021; Iliopoulos et al., 2022). Recently, the CRS method has also been employed to develop a predictive computational model to estimate the permeation of actives in infant skin (Stamatas et al., 2021).

A number of baby wipe formulations contain 2-phenoxyethanol (PE) as a preservative and cetylpyridinium chloride (CPC) as a surfactant with antimicrobial activity. Although several articles have reported the skin permeation of PE, these studies used rodent skin, which is not considered as an appropriate surrogate for human skin. As a comparison, permeation of benzyl alcohol, another phenyl alcohol, has been studied in human skin in vitro. Under finite dose conditions, permeation of benzyl alcohol was evident at 30 min, corresponding to 6-15% of applied dose (Saiyasombati and Kasting, 2003).

Previously we reported the *in vitro* permeation of PE *in vitro* in the presence and absence of CPC using mammalian skin (Rahma et al., 2023). We were motivated to conduct these studies because of the repeated dermal exposure of infants to PE-containing baby wipes and/or other PE-containing personal care products. Skin absorption of PE was evident by 1 h, for both porcine and human tissues. In the present work our goal was to build on the previous *in vitro* findings and probe PE skin uptake *in vivo*. The specific aims of the study were to (i) examine the distribution of PE across the SC *in vivo* using CRS and TS and (ii) to determine any correlations with the previous *in vitro* findings.

2. Materials and Methods

2.1. Materials

CPC and PE were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). High vacuum grease was obtained from Dow Corning (Midland, MI, USA). HPLC grade solvents (acetonitrile, methanol, trifluoroacetic acid, and water) were purchased from Fischer

Scientific (Loughborough, UK). Polyoxyethylene (20) oleyl ether (Brij® O20) was obtained from Sigma-Aldrich (Dorset, UK). Standard D-Squame® 101 tape (2.2 cm in diameter, area 3.8 cm^2) was purchased from CuDerm Corporation (Dallas, TX, USA).

2.2. Preparation of test solutions

The earlier findings from the *in vitro* experiments (Rahma et al., 2023) showed that the highest permeation of PE was observed for a water-PG preparation with CPC (0.2 % w/w). The permeation of PE from this formulation was significantly higher compared with the water-PG preparation without CPC ($p < 0.05$). In addition, these formulations closely simulate the commercial formulations that contain PE and CPC. Therefore, these two preparations were used in the present study. The test solutions were prepared according to the procedures described in the previous work (Rahma et al., 2023).

2.3. Volunteer recruitment

The study was approved by the UCL Research Ethics Committee (Reference number: 24593/001). Healthy adult volunteers were recruited with inclusion and exclusion criteria. Inclusion criteria were as follows: aged 21 to 60 years and with healthy skin. Those with known sensitivity to any of the ingredients and history of any skin disease were excluded from the study. All volunteers completed and signed a questionnaire, confirming their eligibility to participate, as well a consent form. They were provided with a participant information sheet and were instructed not to apply any topical products to the assigned sites (volar forearms) 24 h prior to the experiment.

2.4. Confocal Raman Spectroscopy (CRS) studies

Raman measurements were carried out using a Model 3510 SCA Skin Analyzer Raman spectrometer (RiverD International B.V., Rotterdam, The Netherlands). Two fiber-coupled diode pumped lasers were used, operating at wavelengths of 785 nm and 690 nm.

These wavelengths were used to record spectra in the FP and HWN region, respectively. Intensity calibration was performed by measuring the spectrum of a standard reference material (fused silica) obtained from the National Institute of Standards and Technology (NIST). The signal-to-noise ratio from the calibration measurement was >30. The reference spectra for PE and CPC were acquired in the FP region. Briefly, the Raman spectrum of PE was acquired from neat PE with 100 frames and an exposure time of 10 s per frame. The Raman spectra of human skin were acquired in both the FP and the HWN region for depth profiling and for skin thickness measurements, respectively. Spectral measurements in the FP region were carried out with a 5 s exposure time and 2 µm step size to a final depth of 28 µm. For the HWN measurements, the spectra were taken with 0.5 s exposure times and 4 µm steps to a final depth of 40 µm. For the depth profiling studies, an application site measuring 3.8 cm^2 was delineated on the volar forearm. To prevent the spread of test solutions beyond the designated application site, high vacuum grease was applied around the specified area. Infinite doses, 80 $\mu L/cm^2$, of PE formulations were then applied evenly over the marked area using a pipette and tip, without rubbing. The studies were conducted without any occlusion to simulate in-use conditions. After 60 min, excess formulation was removed using a cotton bud soaked in distilled water. The grease was removed using a cotton bud soaked in 6 % (w/v) Brij® O20 solution in water. This was necessary to prevent contamination of the microscope window of the CRS instrument. Depth profiles of PE were obtained by measuring the Raman spectra of the application site. In order to refine the drug signals, baseline measurements were obtained by recording the spectra of the untreated skin site. All measurements were conducted at a room temperature of 25 ± 2°C and at 55% ± 2% relative humidity. Data were acquired using Skin Tools 2.0 (RiverD International B.V., Rotterdam, The Netherlands). SC thickness was estimated based on the water concentration profiles across the scanned depth, as measured in the HWN region. The depth of each measurement (x) was normalized by the average SC thickness (h) as a function of distance to the skin surface (Herkenne et al., 2006; Mateus et al., 2013).

2.5. Tape stripping

The volunteers were acclimatized for 15 min prior to the experiments. Room conditions were monitored during the study (25 ± 1°C and 55% ± 1% relative humidity). The formulations were applied according to the procedure mentioned in Section 2.4. Briefly, an application site (3.8 cm^2) was delineated and encircled with high vacuum grease. The skin was treated with 80 µL/cm^2 of the formulation for 60 min, and then the excess formulations was removed using a cotton bud soaked in distilled water. The studies were conducted without any occlusion to simulate in-use conditions. The grease was removed using a cotton bud soaked in 6 % (w/v) Brij® O20 solution in water. A standard D-Squame® tape was placed on the designated area and pressure was applied using a pressure instrument (225 g/cm^2 for 10 s). The tape was removed with a constant velocity of removal (5 s for each tape) and placed in a standard D-Squame® Disc Carrier with the adhesive side facing upward. This procedure was repeated in order to obtain twenty consecutive tapes.

2.6. Trans Epidermal Water Loss (TEWL) measurements

The TEWL was measured at the control site using a closed-chamber device (AquaFlux Model AF200, Biox Systems Ltd, London, UK). The probe of the instrument was placed on the skin with a maximum measurement time of 80 s. Baseline TEWL values were obtained by measuring the TEWL 15 min before conducting the TS studies (Section 2.5). During the stripping procedure, TEWL measurements were performed after every five consecutive tape strippings. Data were acquired using AquaFlux software v. 9.3.59 (Biox Systems Ltd, London, UK).

2.7. Protein content measurements

The protein content of the tape strips was measured based on the absorbance of the protein in the tape using a near-infrared densitometer (SquameScan™ 850A, Heiland Electronic GmbH, Wetzlar, Germany) at 850 nm. Prior to measurement, a blank tape strip was used to calibrate the densitometer reading. Protein content was calculated using Equation (1), as described in the published literature (Voegeli et al., 2007).

$$Protein\ Content\ (\mu g/cm^2) = 1.366 * \text{Absorption}\ (\%) - 1.557 \qquad \text{[Eq. 1]}$$

Following the absorbance analysis, the tapes were placed into Eppendorf® tubes. PE and CPC were extracted with methanol-water (85:15) at a constant agitation rate (700 RPM) using an orbital shaker for 12 h. Finally, the tubes were centrifuged at 25 °C at 13,200 rpm for 15 min (Eppendorf 5415R centrifuge, Eppendorf, Hamburg, Germany). The amounts of PE and CPC in the tapes were determined by analysing the supernatant using a validated HPLC method as described elsewhere (Rahma et al., 2023). The extraction method for PE and CPC was validated by spiking TS samples of untreated SC with specified amounts of PE and CPC from one of the test solutions and total active recovery was determined.

2.8. Data treatment and statistical analysis

Data are presented as mean ± SD. Statistical analysis was performed using Origin 8.0 (OriginLab Corp., Northampton, MA). Data were assessed for normal distribution and homogeneity using the Shapiro-Wilk Test and Levene's Test, respectively. Sets of data that met the assumptions of normality and homogeneity were analyzed using a paired t-test. The Wilcoxon test was used for datasets that did not follow the assumptions of normality and/or homogeneity of variance. A probability of $p < 0.05$ was considered to be statistically significant. The correlation assessment was performed using the Pearson correlation coefficient (r^2).

3. Results and discussion

3.1. CRS studies

The Raman spectra of PE in the fingerprint region are shown in Fig. 1 and the peak assignments for the PE spectrum are shown in Table 1. The characteristic peaks observed in the Raman spectrum of PE were in accordance with previously published data (Badawi,

2011). Notably, the ring-breathing mode of PE was observed as a high-intensity peak at 998 cm^{-1}.

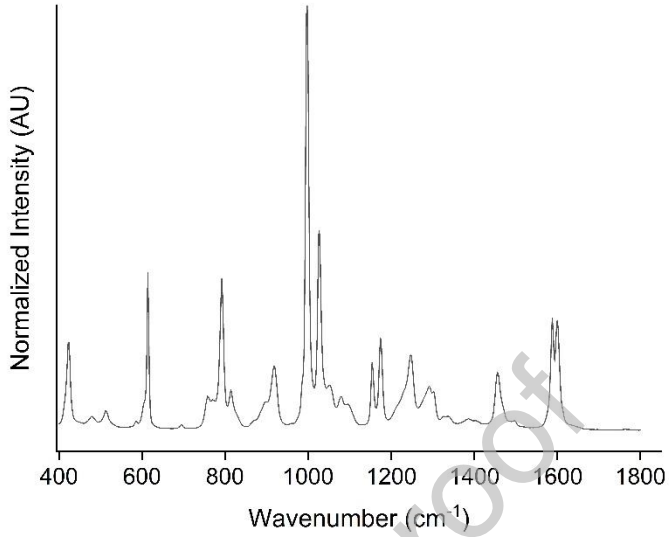

Fig. 1. Raman spectra of PE in the fingerprint region.

The Raman signal of CPC in the formulation was very low, resulting in unreliable measurements for the depth profiling. Given the fact that CPC has a very limited Raman-active vibrational modes, such a finding was not unexpected. This has been considered as one of the current challenges in evaluating topically administered actives using spectroscopic techniques (Raney et al., 2022).

Table 1. Peak assignments for PE in the fingerprint region (Badawi, 2011).

Peak (wavenumber, intensity)	Assignment
423 m	Ring deformation
613 m	Ring deformation
791 m	Ring-O-C deformation

915 w	O-C-C + C-C-O deformation + O-H bending
998 s	Ring breathing
1026 s	Ring deformation + O-C stretching + CH2 twisting + O-H bending
1153 w	C-H (ring) bending
1173 w	C-H (ring) bending
1244 w	CH_2-CH_2 twist + Ring-O + O-C stretching + O-H bending + ring deformation
1290 w	CH_2 twist + C-H (ring) bends + ring deformation
1457 w	C-H (ring) bends + ring deformation
1587 m	C-H (ring) bends + ring deformation
1597 m	C-H (ring) bends + ring deformation

m = medium, s = strong, w = weak

Prior to depth profile measurements, the SC thickness of all volunteers was determined. This was performed to normalize the depth of measurements, minimising the inter-subject variability. The SC thickness of the volunteers ranged from 16.84 ± 2.98 to 20.02 ±3.08 µm, as estimated based on the water concentration profiles in the depth range from 4 to 40 µm below the skin surface. The SC thickness values observed in the present study were in line with the literature (Egawa et al., 2007; Crowther et al., 2008; Mohammed et al., 2012; Dąbrowska et al., 2016). The SC thickness was determined as the distance from the skin surface to the SC-viable epidermis boundary, also as reported in the literature (Pudney et al., 2007; Bielfeldt et al., 2009).

As the CRS probe measured deeper layers of the SC, the absolute Raman intensities decreased with depth. This is the result of increased optical scattering in the skin. Therefore, the signals were normalized to the keratin signals (Caspers et al., 2001). Spectral fitting was applied to all spectra collected through the skin using a least-squares fit algorithm, as reported elsewhere (Caspers et al., 2001). For the depth profiling of PE, two main challenges were present: (i) the low concentration used in the formulations, with a maximum

concentration of 1% in consumer products (European Union Scientific Committee on Consumer Safety, 2016) and (ii) the high rate of permeation – resulting in rapid dose depletion. The latter has also been described in the *in vitro* studies (Rahma et al., 2023).

Fig. 2 shows the signal intensities of PE across the SC after 1 h application of pure PE for a dose of 10 µl/cm^2. PE was detected in the skin up to a normalized depth x/h = 0.6. A steep gradient of intensity was evident from the skin surface to the skin depth of x/h = 0.2. The biphasic behavior observed in the depth profile of neat PE (Fig. 2) indicates the dose depletion of PE formulations, which is the result of (i) finite dose application and (ii) short exposure time. These observations have been reported in an *in vivo* permeation study for caffeine in human skin (Naegel et al., 2011). In another study, *in vivo* skin uptake of propylene glycol, as measured by CRS, showed a similar biphasic profile following a 1 h application under finite dose conditions (Pudney et al., 2007). These results are consistent with our CRS data.

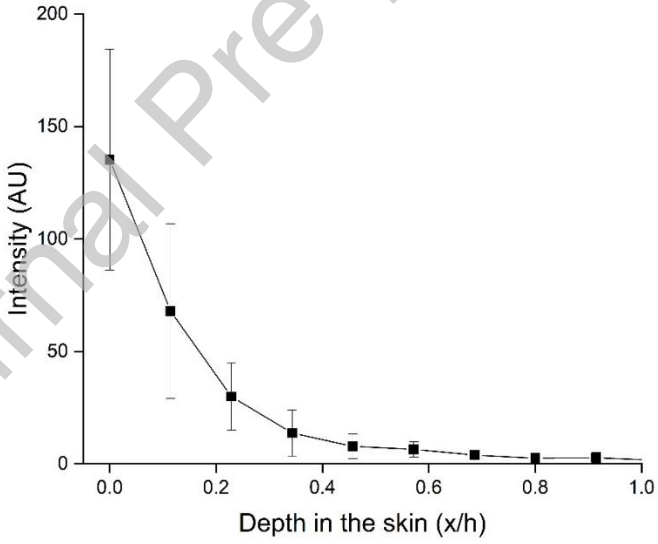

Fig. 2. Depth profile of PE across the SC following 1 h application of neat PE. Mean ± SD, n=5.

Depth profiles of PE in the presence and absence of CPC are shown in Fig. 3. The time point, 1 h, was chosen as PE started to permeate the skin at 1 h *in vitro* (Rahma et al.,

2023). The depth profiles of PE from both solutions exhibited a gradual decrease in the signal intensities, indicating non-steady state diffusion. A higher signal of PE was observed in the upper layers of the SC for the formulation with CPC compared with PE alone. In the lower layers of the SC (0.7≤x/h≤1), the intensity of the PE signals from both preparations were comparable, eventually reaching negligible intensities detected by the instrument. The signal intensities of PE measured by CRS were lower for PE formulations compared with neat PE, suggesting a reduced driving force for skin uptake of PE from the vehicles. We believe the depth profiles of PE may change over time, which is a common observation in CRS studies. Typically, the signal intensity at the skin's surface decreases over time as molecules penetrate deeper (Pudney et al., 2007; Patel et al., 2021). Additionally, PE has been reported to act as a penetration enhancer (Ibrahim and Li, 2009; Ibrahim and Li, 2010). Given that PE may potentially alter the SC organization, further investigation of the depth profile of PE over time is warranted.

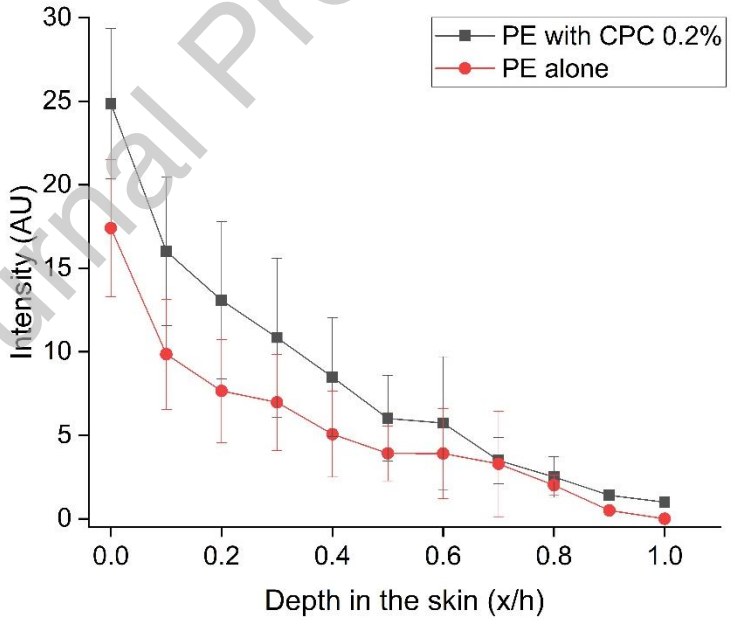

Fig. 3. Depth profile of PE across the SC following 1 h application of PE formulations with and without CPC. Data were obtained from five subjects; mean ± SD.

For further analysis of the depth profiles obtained from CRS studies, the areas under the depth profile curves (AUC) for PE depth profiles were compared for the two preparations (Fig. 4). The AUC value for the formulation with CPC was not significantly different compared with the formulation without CPC ($p > 0.05$). This may reflect inter-subject variability, as can be seen in the depth profiles in Fig. 3.

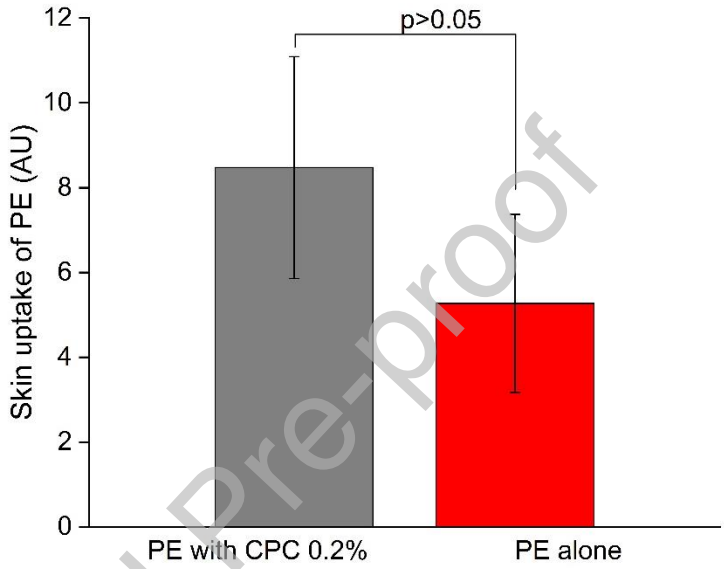

Fig. 4. AUC of PE in the SC following a 1 h application of PE formulations with and without CPC *in vivo*. Mean ± SD of 5 subjects; n = 3-5 replicates per subject.

Notably, the depth profiles and the AUC data were obtained from a 1 h skin exposure to PE formulations. The profiles may change as a function of time. Further experiments with additional time points may permit a more comprehensive understanding of the dynamics of dermal distribution of PE.

3.2. TS studies

The validated extraction method confirmed recovery values of 97.17 ± 4.88 % and 93.52 ± 3.39 % for PE and CPC, respectively. Fig. 5a compares the amounts of PE extracted from the SC using the TS technique following a 1 h application of the two PE formulations. It is evident that the amounts of PE collected for both preparations decreased as the number of strips increased. The amounts of PE recovered in the upper SC layers (as collected by the first 6 tape strips) for the formulation with and without CPC were 26.10 ± 10.69 and 19.29 ± 6.88 µg/cm^2, respectively, and no statistical difference was evident ($p > 0.05$). This result was consistent with the depth profiles of PE for the CRS studies.

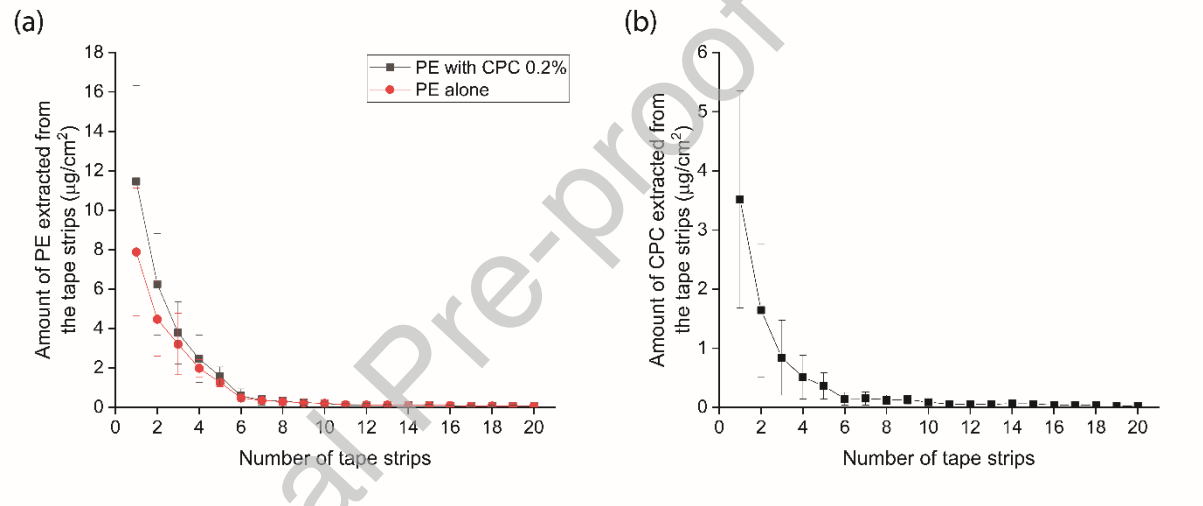

Fig. 5. Amounts of permeants (µg/cm^2), across the tape stripped SC following a 1 h application of test formulations. (a) Amounts of PE collected from the SC for formulations with and without CPC, (b) Amounts of CPC collected from the SC for formulations with CPC. Mean ± SD of 5 subjects.

Fig. 5b shows that less CPC was detected in the TS samples, as the number of tape strips increased. This finding adds insight to the mass balance results from the *in vitro* permeation studies. Previous reports in the literature showed that CPC did not permeate the human skin *in vitro* following a 24 h application of a preparation containing PE and CPC 0.2% (Rahma et al., 2023). However, the mass balance results showed that CPC was retained in the skin, accounting for 7.72 ± 2.33% of the applied dose. In light of the findings

presented in the *in vitro* permeation studies and the *in vivo* TS studies, it may be worth investigating whether CPC could penetrate into the deeper layers of the skin when the upper layers of the SC are disrupted prior to dose application, simulating a damaged skin barrier. This is particularly relevant because the skin of the diaper area is occluded and thus susceptible to overhydration (Rahma and Lane, 2022). Considering that skin cleansing using baby wipes is carried out regularly, the impact of repeated dermal exposure to ingredients contained in baby wipes is a concern.

Removal of the SC by TS causes disruption to the skin barrier (Hadgraft and Lane, 2011). In general, the skin barrier function can be assessed by the TEWL, where elevated TEWL indicates a disrupted skin barrier (Machado et al., 2010; Berardesca et al., 2018). As shown in Fig. 6, TEWL increased significantly ($p < 0.05$) following the TS procedure. The water concentration gradient in the SC is effectively a driving force for the water diffusion from the deeper SC layers towards the skin surface (Xiao and Imhof, 2012). As the SC layers are removed by TS, the outermost SC layer contains a higher percentage of water, which readily evaporates to the outside environment. Consequently, the TEWL will increase compared with the baseline value. In the present study, the TEWL value after removal of 20 tape strips significantly increased from 9.94 ± 1.86 to 16.76 ± 1.98 g/m^{2}h ($p < 0.05$). A study by Bashir et al. (2001) reported an increase in TEWL as a function of the number of strips (volar forearm of six volunteers with D-Squame® tapes) (Bashir et al., 2001). The findings showed that the TEWL values were significantly higher compared with the baseline values ($p < 0.05$) after 30 tape strips were taken. In contrast, with a lower number of tape strips (20 tape strips), the increase in TEWL was reported as not significant ($p > 0.05$) when compared to the baseline values. The authors suggested that the removal of the upper SC layers resulted in insignificant changes in TEWL, while further removal of the deeper SC layers resulted in a significant increase in TEWL (Bashir et al., 2001).

Quantification of the protein content in the TS samples is important as it provides a detailed insight into the penetration depth of the active (Bashir et al., 2001; Jacobi et al., 2003; Löffler et al., 2004; Lademann et al., 2009; Klang et al., 2011). Clearly, the amounts of active collected by each tape strip ultimately depend on the amount of SC removed, which is not always consistent. This is associated with the high variability in SC cohesion (Fluhr and

Elias, 2002). The SC cohesion can be defined as the corneocytes' resistance to removal from the adjacent SC layers (Fluhr and Elias, 2002). The amount of SC removed by the tape may be estimated by the protein content in the TS samples.

In the present study, the protein content in the 20 tapes collected after 1 h exposure to PE formulations is shown in Fig. 6. Overall, the SC protein content decreased as more TS samples were taken, indicating that less protein was removed from the deeper layers of the SC. This is associated with higher inter-corneocyte cohesion in the lower layers of the SC (King et al., 1979). Taken together with the depth profiles of PE and CPC (Fig. 5a-b), the amounts of the two compounds collected on the tapes as well as the protein content (Fig. 6), progressively decreased as the number of tapes increased. Therefore, it is possible that lower amounts of PE and CPC recovered from the deeper layers of the skin were in part due to lower removal of the SC, particularly from the 10^{th} strip. Other researchers have also reported a decreasing amount of protein recovered as the number of strips increased, indicating the stronger intercellular cohesion in the lower layers of the SC (King et al., 1979; Breternitz et al., 2007; Voegeli et al., 2007; Mohammed et al., 2011; Mohammed et al., 2012).

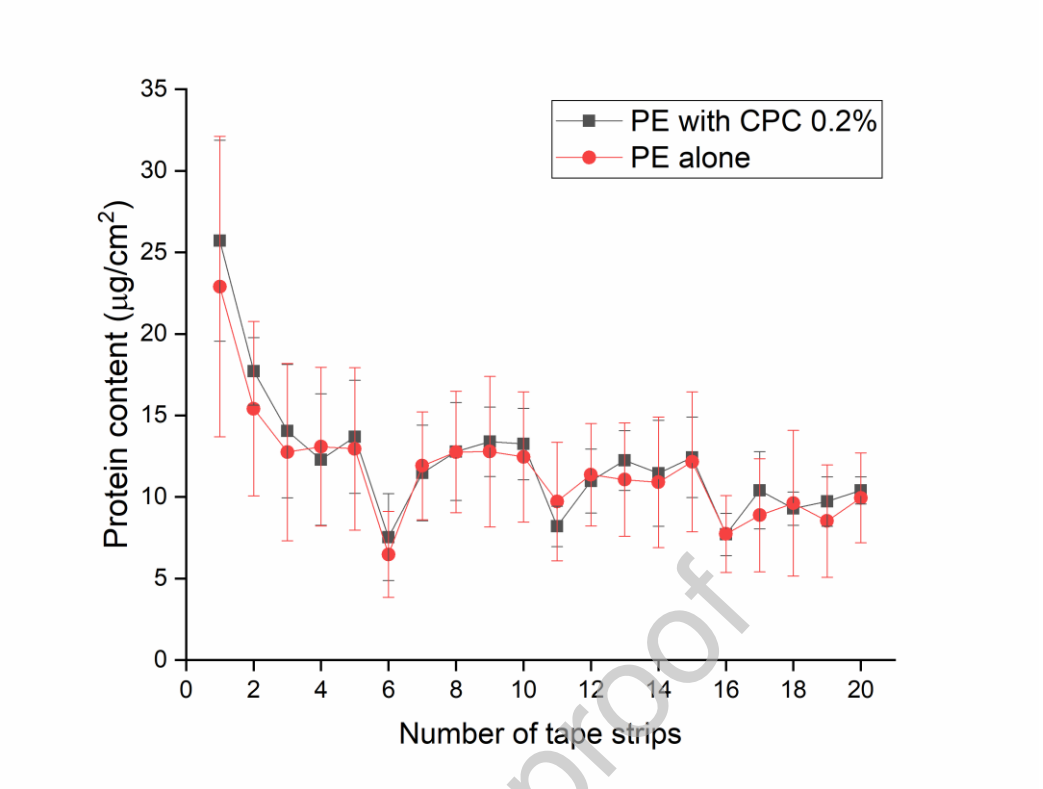

Fig. 6. Amount of SC protein removed with increasing number of tape strips following a 1 h application of PE formulations with and without CPC. Mean ± SD of 5 subjects.

Fig. 7 shows the amounts of PE and CPC recovered from the tape strips, normalized as the amount of active (µg) per amount of protein (µg). The depth profiles showed that both PE and CPC amounts progressively decreased as the number of tape strips increased. The cumulative amounts of PE recovered in the upper SC layers per amount of protein (collected by the first 6 tape strips) for the formulation with and without CPC were 1.96 ± 0.68 and 1.84 ± 1.20 µg/µg protein, respectively. No statistical difference was evident when comparing these two formulations ($p > 0.05$).

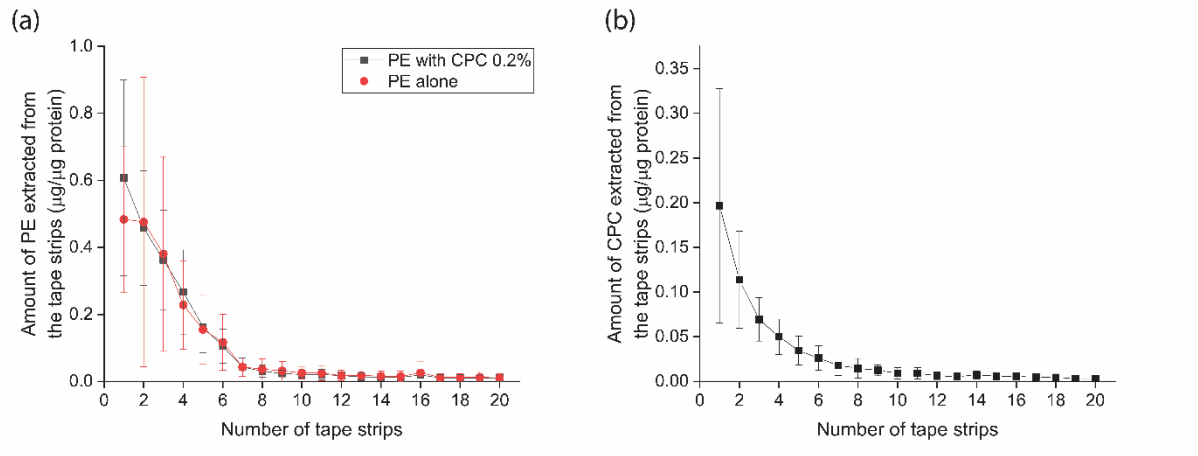

Fig. 7. Amounts of PE and CPC for the tape stripped SC following 1 h application of formulations, normalized to protein content (µg/µg). (a) PE, (b) CPC. Mean ± SD of 5 subjects.

Quantification of the SC protein collected in each tape may also provide an estimation of the total thickness of SC removed (Mao et al., 2012; Mohammed et al., 2012). Mohammed et al. observed a correlation between the amount of SC protein extracted and the weight of SC removed in each tape (Mohammed et al., 2012) as shown in Equation (2):

$$Protein\ content\ \left(\frac{\mu g}{cm^2}\right) = 0.420 \times SC\ weight\ \left(\frac{\mu g}{cm^2}\right) \qquad \text{[Eq. 2]}$$

Assuming that the SC density is about 1 g/cm^3, as reported in the literature (Anderson and Cassidy, 1973; Kalia et al., 2001), the SC thickness (µm) may be determined based on the SC weight per unit area (µg/cm^2) and the SC density (g/cm^3). The cumulative SC thickness removed from each tape was then calculated. Fig. 8 shows that PE permeated to a SC depth of 5.82 ± 0.58 µm and 5.55 ± 1.71 µm for the preparation containing CPC and without CPC, respectively. The average SC thickness is reportedly about 15 µm, and this value has been generally accepted for analysis of *in vivo* permeation (Holbrook and Odland, 1974; Pirot et al., 1997; Caspers et al., 2019). Assuming this value for SC thickness, it may be

estimated that PE penetrated to a depth of 38.84% and 37.04% of the SC for the preparation with and without CPC, respectively. These depths of penetration did not differ significantly between the two preparations ($p > 0.05$). In contrast, the CRS data showed that PE was detected up to a depth of 60% of the SC, equivalent to $x/h = 0.6$ (Fig. 2). This underlines the main advantage of the CRS technique, where the signal intensity of active measured by the instrument is not affected by the SC protein removal. In addition, CRS enables depth profiling in a stepwise and uniform increment for all subjects (2 µm step size to a final depth of 28 µm).

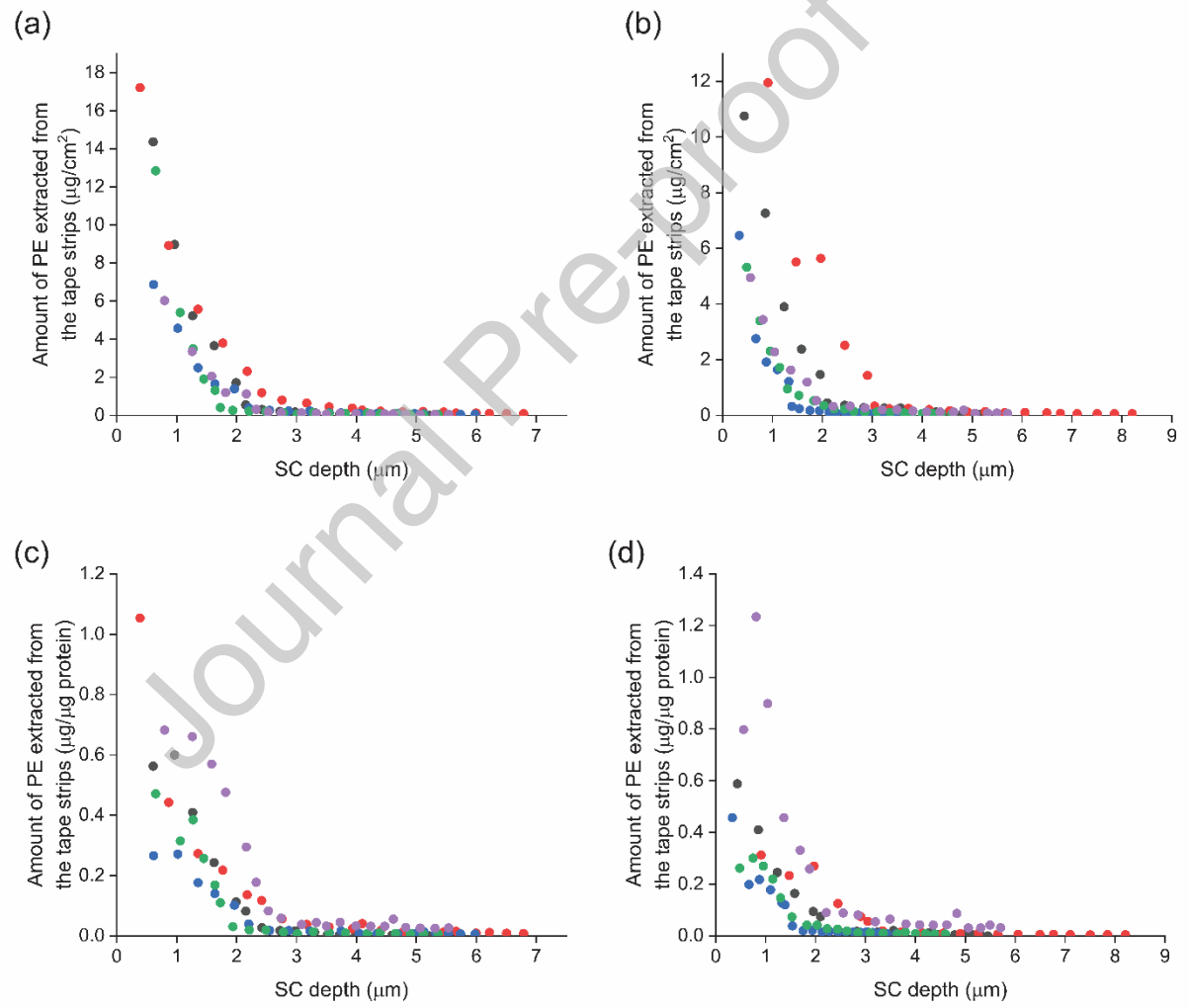

Fig. 8. Amounts of PE across the tape stripped SC following 1 h application of formulations. (a) PE in the presence of CPC, expressed as µg/cm^2, (b) PE alone, expressed as

μg/cm^2, (c) PE in the presence of CPC, normalized to protein content (μg/μg), (d) PE alone, normalized to protein content (μg/μg). n=5 subjects.

3.3. Comparative evaluation of the permeation data

CRS and TS methods in this study provided complementary information to the *in vitro* data regarding the skin permeation of PE. In previous *in vitro* permeation studies, the cumulative amounts of PE permeated through human skin at 24 h (Q_{24}) from the formulations with and without CPC were 92.46 ± 9.34 and 80.54 ± 6.33 μg/cm^2, respectively (Rahma et al., 2023). In Fig. 9, Q_{24} values were plotted against the relevant permeation parameters *in vivo* from the present study, namely AUC for PE as measured by CRS (AUC), and cumulative amount of PE recovered from the tape strips (Q_{TS}). Correlation for Q_{24} and AUC was indicated by Pearson's r^2 value of 0.97. A positive correlation between *in vitro* permeation data and CRS data has been reported in the literature (Mohammed et al., 2014; Iliopoulos et al., 2020; Zhang et al., 2021; Patel et al., 2021). Mohammed et al. (2014) and Zhang et al. (2021) reported a correlation between the cumulative permeation of niacinamide *in vitro* and the signal intensity of niacinamide in the SC *in vivo* as measured by CRS ($r^2 > 0.95$). Additionally, Iliopoulos et al. (2020) reported the first *in vivo* quantitative CRS studies for niacinamide from various formulations. These researchers found an excellent correlation between the amounts of niacinamide measured per unit skin protein at a skin depth of 2 μm and the cumulative permeation of niacinamide *in vitro* ($r^2 = 0.98$). More recently, Patel et al. (2021) reported an *in vitro-in vivo* correlation for ibuprofen. In the study, the cumulative amount of ibuprofen permeated *in vitro* and the total amount of ibuprofen penetrated the stratum corneum (SC) i*n vivo*, as measured using CRS, exhibited a correlation ($r^2 = 0.90$).

When comparing the *in vitro* permeation and TS data in the present study, a correlation between Q_{24} and Q_{TS} for PE was observed, with a corresponding r^2 value of 0.95. This positive correlation was in line with previously reported findings (Zhang et al., 2021). The authors found that the amounts of niacinamide that permeated through human skin *in vitro* correlated with the amounts of niacinamide recovered from TS ($r^2 = 0.86$).

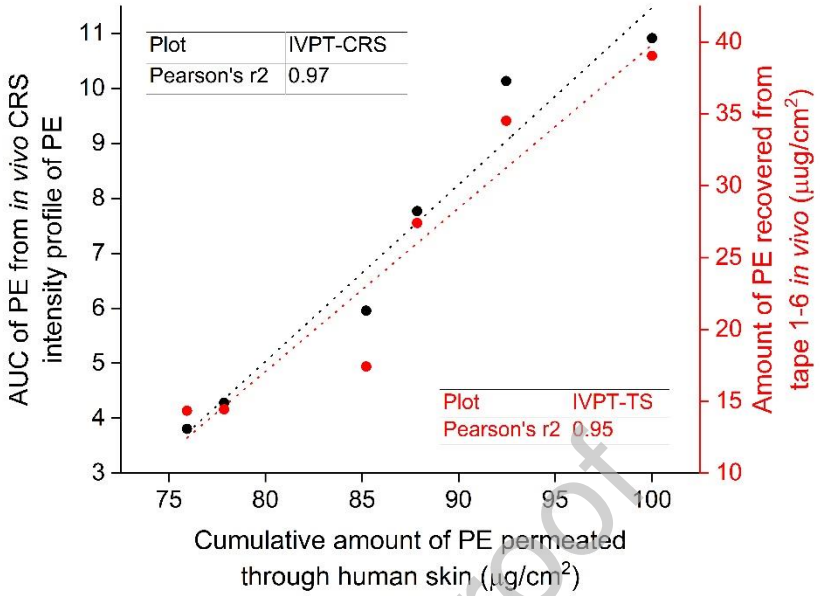

Fig. 9. Correlation between *in vitro* and *in vivo* permeation data for PE.

Notably, for these studies, both CRS and TS techniques demonstrated limitations in assessing the distribution of PE and CPC in the skin *in vivo*, primarily related to the Raman signal intensities of compounds under investigation and the amount of SC removed by TS.

The *in vivo* experiments in the present study were conducted with an exposure period of 1 h under infinite dose conditions. Clearly, investigating PE distribution in the skin *in vivo* under finite dose conditions is challenging because there is a risk of low signal-to-noise ratio associated with the dose depletion. More importantly, commercial baby wipe formulations contain PE at a concentration of not more than 1% w/v (European Union Scientific Committee on Consumer Safety, 2016). However, the practice of wiping the diaper area is carried out regularly. Therefore, the dosing condition in the present study may be a reasonable approximation to the actual in-use exposure to PE from baby wipes. This approach has been used in a permeation study to simulate repeated application of two sunscreen ingredients: octyl methoxycinnamate and butylmethoxydibenzoylmethane (Montenegro et al., 2018). In that study, *in vitro* permeation studies of formulations

containing these actives were carried out under infinite dose conditions (20 mg/cm^2) in human skin, with an assumption that sunscreen products are applied multiple times over an extended period of time.

Considering the points mentioned above, it is worth noting that *in vitro* studies remain of great value to the personal care and pharmaceutical sectors. From an ethical standpoint, *in vitro* experiments offer several advantages, including extended study periods and the possibility of simulating repeated exposure. In addition to ethical considerations, there is a wide range of analytical techniques available for sample analysis, including High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Liquid Chromatography coupled with Mass Spectrometry (LC/MS). These techniques have advanced with reference to sensitivity and limits of detection in recent years, features that are extremely beneficial for analysis of compounds that do not exhibit strong Raman signals.

4. Conclusions

To our knowledge, this is the first study to report the uptake of PE in human skin *in vivo*. In the previous work, the *in vitro* data in human skin showed that PE started to permeate the human skin 1 h after application. The findings in this *in vivo* experiment supported the *in vitro* results, confirming the penetration of PE in the SC after a 1 h application of the formulations. Analysis of CRS and TS data revealed that the inclusion of 0.2% CPC in the formulation did not result in a significant increase in skin uptake of PE ($p > 0.05$) compared to the formulation without CPC following a 1 h application of PE formulations. Clearly, the use of CRS for assessing skin penetration is not applicable for all compounds since the measurement relies on sufficient signal intensities. The challenge of precisely controlling the amounts of SC removed by TS was evident in the present study. Despite the limitations of CRS and TS, the findings from this *in vivo* experiment add further value to our understanding of PE distribution across the SC. The *in vitro-in vivo* correlation observed in the present study is also encouraging, providing further support for the use of CRS as a non-invasive method for assessing topical skin formulations *in vivo*.

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CRediT authorship contribution statement

Annisa Rahma: Conceptualization, Data curation, Methodology, Investigation, Formal analysis, Project administration, Validation, Visualization, Writing – original draft. Jingyi Gu: Data curation, Investigation. Majella E. Lane: Conceptualization, Resources, Supervision, Writing – review & editing.

References

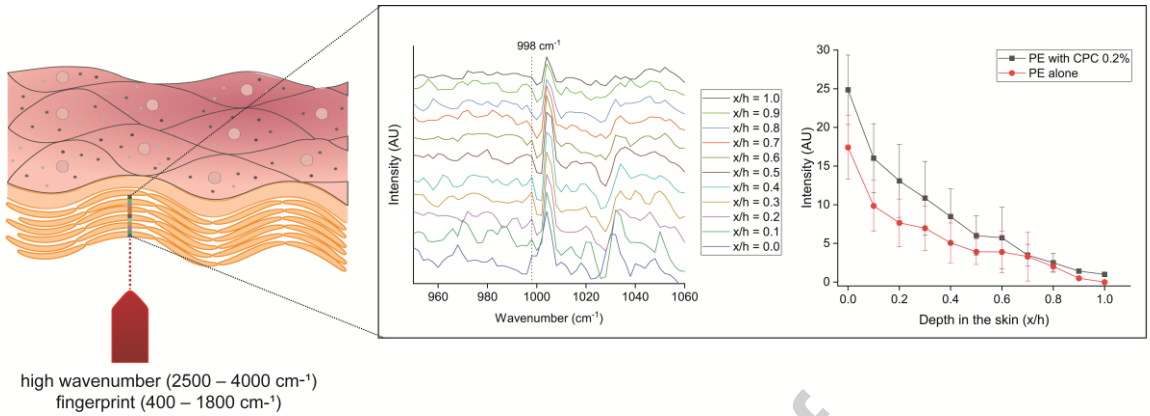
Anderson, R.L., Cassidy, J.M., 1973. Variations in Physical Dimensions and Chemical Composition of Human Stratum Corneum. J. Invest. Dermatol. 61, 30-32.
Badawi, H.M., 2011. Vibrational spectra and assignments of 2-phenylethanol and 2-phenoxyethanol. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 82, 63-68.
Bashir, S.J., Chew, A.L., Anigbogu, A., Dreher, F., Maibach, H.I., 2001. Physical and physiological effects of stratum corneum tape stripping. Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI) 7, 40-48.
Berardesca, E., Loden, M., Serup, J., Masson, P., Rodrigues, L.M., 2018. The revised EEMCO guidance for the in vivo measurement of water in the skin. Skin Res. Technol. 24, 351-358.
Bielfeldt, S., Bonnier, F., Byrne, H.J., Chourpa, I., Dancik, Y., Lane, M.E., Lunter, D.J., Munnier, E., Puppels, G., Tfayli, A., Ziemons, E., 2022. Monitoring dermal penetration and permeation

kinetics of topical products; the role of Raman microspectroscopy. TrAC Trends in Analytical Chemistry 156, 116709.
Bielfeldt, S., Schoder, V., Ely, U., Van Der Pol, A., De Sterke, J., Wilhelm, K.-P., 2009. Assessment of human stratum corneum thickness and its barrier properties by in-vivo confocal Raman spectroscopy. Int. J. Cosmet. Sci. 31, 479-480.
Breternitz, M., Flach, M., Präßler, J., Elsner, P., Fluhr, J.W., 2007. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping; a randomized, controlled study. Br. J. Dermatol. 156, 231-240.
Caspers, P.J., Bruining, H.A., Puppels, G.J., Lucassen, G.W., Carter, E.A., 2001. In Vivo Confocal Raman Microspectroscopy of the Skin: Noninvasive Determination of Molecular Concentration Profiles. J. Invest. Dermatol. 116, 434-442.
Caspers, P.J., Lucassen, G.W., Bruining, H.A., Puppels, G.J., 2000. Automated depth-scanning confocal Raman microspectrometer for rapid in vivo determination of water concentration profiles in human skin. J. Raman Spectrosc. 31, 813-818.
Caspers, P.J., Nico, C., Bakker Schut, T.C., de Sterke, J., Pudney, P.D., Curto, P.R., Illand, A., Puppels, G.J., 2019. Method to quantify the in vivo skin penetration of topically applied materials based on confocal Raman spectroscopy. Translational Biophotonics 1, e201900004.
Crowther, J.M., Sieg, A., Blenkiron, P., Marcott, C., Matts, P.J., Kaczvinsky, J.R., Rawlings, A.V., 2008. Measuring the effects of topical moisturizers on changes in stratum corneum thickness, water gradients and hydration in vivo. British journal of dermatology (1951) 159, 567-577.
Dąbrowska, A.K., Adlhart, C., Spano, F., Rotaru, G.M., Derler, S., Zhai, L., Spencer, N.D., Rossi, R.M., 2016. In vivo confirmation of hydration-induced changes in human-skin thickness, roughness and interaction with the environment. Biointerphases 11, 031015.
Dupuis, D., Rougier, A., Roguet, R., Lotte, C., Kalopissis, G., 1984. In Vivo Relationship Between Horny Layer Reservoir Effect and Percutaneous Absorption in Human and Rat. J. Invest. Dermatol. 82, 353-356.
Egawa, M., Hirao, T., Takahashi, M., 2007. In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy. Acta Derm. Venereol. 87, 4-8.
European Union Scientific Committee on Consumer Safety, 2016. Opinion on Phenoxyethanol, in: European Commission Health and Food Safety Directorate C: Public Health (Ed.).
Fluhr, J.W., Elias, P.M., 2002. Stratum corneum pH: Formation and Function of the 'Acid Mantle'. Exogenous Dermatology 1, 163-175.
Hadgraft, J., Lane, M.E., 2011. Skin: the ultimate interface. Phys. Chem. Chem. Phys. 13, 5215-5222.
Herkenne, C., Naik, A., Kalia, Y.N., Hadgraft, J., Guy, R.H., 2006. Pig Ear Skin ex Vivo as a Model for in Vivo Dermatopharmacokinetic Studies in Man. Pharm. Res. 23, 1850-1856.
Holbrook, K.A., Odland, G.F., 1974. Regional Differences in the Thickness (Cell Layers) of the Human Stratum Corneum: An Ultrastructural Analysis. J. Invest. Dermatol. 62, 415-422.
Ibrahim, S.A., Li, S.K., 2009. Effects of chemical enhancers on human epidermal membrane: Structure-enhancement relationship based on maximum enhancement (E(max)). J. Pharm. Sci. 98, 926-944.

Ibrahim, S.A., Li, S.K., 2010. Chemical enhancer solubility in human stratum corneum lipids and enhancer mechanism of action on stratum corneum lipid domain. Int. J. Pharm. 383, 89-98.
Iliopoulos, F., Caspers, P.J., Puppels, G.J., Lane, M.E., 2020. Franz Cell Diffusion Testing and Quantitative Confocal Raman Spectroscopy: In Vitro-In Vivo Correlation. Pharmaceutics 12, 887.
Iliopoulos, F., Goh, C.F., Haque, T., Rahma, A., Lane, M.E., 2022. Dermal Delivery of Diclofenac Sodium—In Vitro and In Vivo Studies, Pharmaceutics.
Iliopoulos, F., Tang, C.F., Li, Z., Rahma, A., Lane, M.E., 2023. Confocal Raman Spectroscopy for Assessing Bioequivalence of Topical Formulations, Pharmaceutics.
Jacobi, U., Meykadeh, N., Sterry, W., Lademann, J., 2003. Effect of the vehicle on the amount of stratum corneum removed by tape stripping. JDDG: Journal der Deutschen Dermatologischen Gesellschaft 1, 884-889.
Kalia, Y.N., Alberti, I., Naik, A., Guy, R.H., 2001. Assessment of Topical Bioavailability in vivo: The Importance of Stratum corneum Thickness. Skin Pharmacol. Physiol. 14(suppl 1), 82-86.
King, C.S., Barton, S.P., Nicholls, S., Marks, R., 1979. The change in properties of the stratum corneum as a function of depth. Br. J. Dermatol. 100, 165-172.
Klang, V., Schwarz, J.C., Hartl, A., Valenta, C., 2011. Facilitating in vitro tape stripping: application of infrared densitometry for quantification of porcine stratum corneum proteins. Skin Pharmacol. Physiol. 24, 256-268.
Lademann, J., Jacobi, U., Surber, C., Weigmann, H.J., Fluhr, J.W., 2009. The tape stripping procedure – evaluation of some critical parameters. Eur. J. Pharm. Biopharm. 72, 317-323.
Löffler, H., Dreher, F., Maibach, H.I., 2004. Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal. Br. J. Dermatol. 151, 746-752.
Machado, M., Hadgraft, J., Lane, M., 2010. Assessment of the variation of skin barrier function with anatomic site, age, gender and ethnicity. Int. J. Cosmet. Sci. 32, 397-409.
Mao, G., Flach, C.R., Mendelsohn, R., Walters, R.M., 2012. Imaging the distribution of sodium dodecyl sulfate in skin by confocal Raman and infrared microspectroscopy. Pharm. Res. 29, 2189-2201.
Mateus, R., Abdalghafor, H., Oliveira, G., Hadgraft, J., Lane, M.E., 2013. A new paradigm in dermatopharmacokinetics – Confocal Raman spectroscopy. Int. J. Pharm. 444, 106-108.
Mateus, R., Moore, D.J., Hadgraft, J., Lane, M.E., 2014. Percutaneous absorption of salicylic acid – in vitro and in vivo studies. Int. J. Pharm. 475, 471-474.
Mohammed, D., Matts, P.J., Hadgraft, J., Lane, M.E., 2011. Depth profiling of stratum corneum biophysical and molecular properties. Br. J. Dermatol. 164, 957-965.
Mohammed, D., Matts, P.J., Hadgraft, J., Lane, M.E., 2014. In Vitro–In Vivo Correlation in Skin Permeation. Pharm. Res. 31, 394-400.
Mohammed, D., Yang, Q., Guy, R.H., Matts, P.J., Hadgraft, J., Lane, M.E., 2012. Comparison of gravimetric and spectroscopic approaches to quantify stratum corneum removed by tape-stripping. Eur. J. Pharm. Biopharm. 82, 171-174.
Montenegro, L., Turnaturi, R., Parenti, C., Pasquinucci, L., 2018. In Vitro Evaluation of Sunscreen Safety: Effects of the Vehicle and Repeated Applications on Skin Permeation from Topical Formulations, Pharmaceutics.

Naegel, A., Hahn, T., Schaefer, U.F., Lehr, C.-M., Heisig, M., Wittum, G., 2011. Finite dose skin penetration: a comparison of concentration-depth profiles from experiment and simulation. Computing and Visualization in Science 14, 327-339.
Organisation for Economic Cooperation and Development, 2019. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 156 - Guidance Notes on Dermal Absorption.
Organization for Economic Cooperation Development, 2004. Test No. 428: Skin absorption: *in vitro* method, Section 4. OECD Publishing, Paris.
Patel, A., Iliopoulos, F., Caspers, P.J., Puppels, G.J., Lane, M.E., 2021. In Vitro–In Vivo Correlation in Dermal Delivery: The Role of Excipients. Pharmaceutics 13, 542.
Pirot, F., Kalia, Y.N., Stinchcomb, A.L., Keating, G., Bunge, A., Guy, R.H., 1997. Characterization of the permeability barrier of human skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 94, 1562-1567.
Pudney, P.D.A., Mélot, M., Caspers, P.J., van der Pol, A., Puppels, G.J., 2007. An In Vivo Confocal Raman Study of the Delivery of Trans-Retinol to the Skin. Applied Spectroscopy 61, 804-811.
Rahma, A., Lane, M.E., 2022. Skin Barrier Function in Infants: Update and Outlook. Pharmaceutics 14.
Rahma, A., Lane, M.E., Sinkó, B., 2023. A comparative study of the in vitro permeation of 2-phenoxyethanol in the skin PAMPA model and mammalian skin. Int. J. Pharm. 635, 122692.
Raney, S.G., Ghosh, P., Ramezanli, T., Lehman, P.A., Franz, T.J., 2022. Cutaneous Pharmacokinetic Approaches to Compare Bioavailability and/or Bioequivalence for Topical Drug Products. Dermatol. Clin. 40, 319-332.
Rougier, A., Dupuis, D., Lotte, C., Roguet, R., Wester, R.C., Maibach, H.I., 1986. Regional variation in percutaneous absorption in man: measurement by the stripping method. Arch. Dermatol. Res. 278, 465-469.
Saiyasombati, P., Kasting, G.B., 2003. Disposition of Benzyl Alcohol After Topical Application to Human Skin in vitro. J. Pharm. Sci. 92, 2128-2139.
Stamatas, G.N., Bensaci, J., Greugny, E., Kaur, S., Wang, H., Dizon, M.V., Cork, M.J., Friedman, A.J., Oddos, T., 2021. A Predictive Self-Organizing Multicellular Computational Model of Infant Skin Permeability to Topically Applied Substances. J. Invest. Dermatol. 141, 2049-2055.e2041.
Voegeli, R., Heiland, J., Doppler, S., Rawlings, A.V., Schreier, T., 2007. Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry. Skin Res. Technol. 13, 242-251.
Xiao, P., Imhof, R.E., 2012. Two dimensional finite element modelling for dynamic water diffusion through stratum corneum. Int. J. Pharm. 435, 88-92.
Zhang, Y., Kung, C.-P., Iliopoulos, F., Sil, B.C., Hadgraft, J., Lane, M.E., 2021. Dermal Delivery of Niacinamide—In Vivo Studies. Pharmaceutics 13.

Graphical abstract



Declaration of Competing Interest

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.