Solvent and Crystallization Effects on the Dermal Absorption of Hydrophilic and Lipophilic Compounds

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ABSTRACT

This study probes the mechanisms by which volatile solvents (water, ethanol) and a nonionic surfactant (Triton X-100™) influence the skin permeation of dissolved solutes following deposition of small doses onto unoccluded human skin. A secondary objective was to sharpen guidelines for the use of these and other simple solvent systems for dermal safety testing of cosmetic ingredients at finite doses. Four solutes were studied – niacinamide, caffeine, testosterone and geraniol – at doses close to that estimated to saturate the upper layers of the stratum corneum. Methods included tensiometry, visualization of spreading on skin, polarized light microscopy and in vitro permeation testing using radiolabeled solutes. Ethanol, aqueous ethanol and dilute aqueous Triton solutions all yielded surface tensions below 36 mN/m, allowing them to spread easily on the skin, unlike water (72.4 mN/m) which did not spread. Deposition onto skin of niacinamide (32 $\mu g \cdot cm^{-2}$) or caffeine (3.2 $\mu g \cdot cm^{-2}$) from water and ethanol led to crystalline deposits on the skin surface, whereas the same amounts applied from aqueous ethanol and 2% Triton did not. Skin permeation of these compounds was inversely correlated to the extent of crystallization. A separate study with caffeine showed the absence of a dose-related skin permeability increase with Triton. Permeation of testosterone (8.2 $\mu g \cdot cm^{-2}$) was modestly increased when dosed from aqueous ethanol versus ethanol. Permeation of geraniol (2.9 $\mu g \cdot cm^{-2}$) followed the order aqueous ethanol > water ~ 2% Triton >> ethanol and was inversely correlated with evaporative loss. We conclude that, under the conditions tested, aqueous ethanol and Triton serve primarily as deposition aids and do not substantially disrupt stratum corneum lipids. Implications for the design of in vitro skin permeability tests are discussed.

Keywords: crystallization; percutaneous absorption; solvent effects; skin permeability; topical

delivery

Highlights

- Solvent deposition of dissolved solutes on human skin shows that spreading and evaporation rates and (possibly) osmosis govern dermal delivery.
- At low doses, aqueous ethanol or dilute nonionic surfactants improve delivery versus water or ethanol alone without impacting the stratum corneum lipid barrier.
- Authors recommend these excipients for finite dose testing of pharmaceutical and cosmetic ingredients.

Abbreviations: ANOVA – analysis of variance; CE – Cosmetics Europe; DD – dermal delivery; DMI – dimethyl isosorbide; ESD – extreme studentized deviate; EtOH – ethanol; GS – glass slides; HEM – human epidermal membrane; IPM – isopropyl myristate; IVPT – in vitro permeation test; MD – molecular dynamics; MW – molecular weight; PBS – phosphate-buffered saline; RF – receptor fluid; RSE – relative standard error; SC – stratum corneum; SFT – surface tension; SGS – silanized glass slides; TC -- Transcutol®; Z – test statistic

Introduction

Much has been written regarding the impact of volatile solvents on percutaneous absorption including their effect on crystallization of topically-applied compounds on and in the skin – see Hadgraft and Lane[1] for a recent review. In general, it may be stated that solute precipitation on and in the upper skin layers slows permeation, and that gradual re-dissolution of the precipitate can lead to sustained delivery if the precipitate is not washed or rubbed off. It is evident that multiple factors may come into play in quantitatively determining the outcome – the dose and volatility of the solvent,[2] the dose of the compound relative to the skin's capacity to absorb it,[3] the ability of the formulation to wet and spread on the skin,[4] the impact of the solvent on stratum corneum barrier function,[5,6] and the solid state properties of the solute including nucleation kinetics.[1] For weak electrolytes applied to skin in various ionization states, the buffer capacity of the dose solution relative to that of the skin plays a profound role in absorption.[7]

Hadgraft and Lane presented a historical overview of crystallization effects focused, in particular, upon technological advances in *preventing* crystallization in order to improve drug delivery.[1] An even larger body of literature focuses on solvent enhancement of skin permeation. Gupta and coworkers recently summarized work in this area with respect to ethanol and presented five mechanisms by which ethanol may enhance skin permeation. They may be captured briefly as lipid extraction, lipid fluidization, alteration of stratum corneum (SC) protein conformation, co-permeation of drug with alcohol and enhancement of drug solubility in SC lipids.[8] In the same article, the authors went on to describe a molecular dynamics (MD) study of a model SC lipid bilayer surrounded by ethanol/water mixtures having a wide range of ethanol content. The simulations showed that ethanol mole fractions (x) in the range $0.2 < x < 0.6$ led to extraction of free fatty acids from the bilayer and that ceramides were also extracted for $x > 0.6$.

Values of $x > 0.8$ led to nearly complete disruption of the bilayer. Thus, their work supports a lipid extraction mechanism for skin barrier disruption. These findings will be compared later with contrasting experimental results from the present study and others in the literature. Is ethanol really that damaging to skin?

In this study we focused on the skin disposition of small doses of nonelectrolytes applied to skin in water, dilute surfactant solutions, ethanol and aqueous ethanol. Two hydrophilic and two lipophilic permeants were tested. Several experimental approaches were employed – deposition and crystallization on glass slides and human skin, spreading and evaporation from glass slides, and in vitro permeation through human skin. We were particularly interested whether the results could help to explain the pattern of absorption observed in the recent Cosmetics Europe in vitro skin permeation study in which small doses of cosmetic-relevant compounds were applied to human skin in either phosphate-buffered saline, acetone or ethanol.[9] Several research groups, including our own, have offered different interpretations of this study.[10-12] The results of the analysis, combined with our earlier work on weak electrolytes,[7] show that spreading or lack thereof of the formulation on the skin surface, followed by crystallization of either the neutral solute or a salt thereof, plays a significant role in the skin disposition of the solvent-deposited chemicals. Solvents that spread easily on the skin promote rapid penetration of dissolved solutes into the upper layers of the SC leading to higher dermal absorption. For small solvent doses that evaporate quickly, this can happen without a concomitant increase in skin permeability.

Materials and methods

Materials

Radiochemicals (carbonyl-^{14}C-nicotinamide, 1-methyl-^{14}C-caffeine, 4-^{14}C-testosterone and 3-^{14}C-geraniol) were purchased from American Radiolabeled Chemicals (St. Louis, MO) at a

specific activity of 10-55 mCi $mmol^{-1}$. Radiochemical purity was stated by the manufacturer to be 99%. Tritiated water (^3H_2O) and Solvable™ tissue solubilizer were obtained from PerkinElmer (Waltham, MA). Ultima Gold liquid scintillation cocktail was obtained from ThermoFisher Scientific (Hampton, NH). Unlabeled samples of these chemicals (reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO). Deionized water (17.8 MΩ·cm) was obtained from a Millipore system. Organic solvents (HPLC grade), Dulbecco's phosphate buffered saline (Ca free) and Igepal CA-360 were obtained from Sigma-Aldrich. Triton X-100 was purchased from Integra Chemical Co. (Kent, WA). Mild hand soap (Wash 20, Creative Laboratories, Egan, MN) was purchased at a local grocery store.

Split-thickness human cadaver skin was purchased from New York Firefighters' Skin Bank (New York, NY). The skin was stored at −80℃ for up to three months. Prior to use, it was thawed by immersion in PBS buffer (pH 7.4 PBS + 0.02% w/v sodium azide). For some experiments, human epidermal membrane (HEM) was prepared by immersing the skin in PBS buffer at 60°C for one minute, then laying it on aluminum foil. The epidermis layer was peeled off, covered and stored at −20℃ prior to use.

Test solutes and solvents

Four test solutes were studied, two of which were hydrophilic (niacinamide, caffeine) and two lipophilic (testosterone, geraniol). Physical properties of these solutes are shown in Table 1.

Solvents included water, ethanol (EtOH), various EtOH:water mixtures and dilute nonionic surfactant mixtures in water [Triton X-100 (octoxynol) at several concentrations and Igepal CA-630 (octylphenoxy polyethoxyethanol)]. Triton was chosen as a nonionic surfactant with excellent solubilizing power for both aliphatic and aromatic compounds. When we realized that

Table 1

Physical properties of test solutes. Values are from PubChem unless otherwise noted.

Permeant	MW, g/mol	mp, °C	log K_{oct}[a]	P_{vp}[b], 25°C mmHg	Solubilities, g/L			
					Water		Ethanol	70% EtOH
					25°C	32°C	32°C	32°C
Niacinamide	122.12	130	−0.37[c]; −1.39[c]	8.07E-05	500	534[c]	150[g]	220[h]
Caffeine	194.19	238	−0.07	7.33E-09	21.0[d]	26.8[d]	4.49[f]	7.8[h]
Testosterone	288.4	155	3.32	2.23E-08	0.234	0.278[e]	106[f]	18[h]
Geraniol	152.2	−15	3.56	3.00E-02	-	0.349[f]	151[f]	20[h]

[a]logarithm of octanol:water partition coefficient
[b]Vapor pressure
[c]Ref. 13. The two values of log K_{oct} refer to dilute and saturated solutions, respectively.
[d]Estimated based on Arrhenius analysis of data in Ref. 14
[e]Estimated as $1.19 \times S_w$ (25°C)
[f]Ref. 15
[g]Estimated based on solubility data in other small alcohols in Refs. 16 and 17
[h]Estimated based on loglinear cosolvent approximation, discussed in the Supplementary Material

Triton was recognized as a cytotoxic agent with mild skin penetration enhancement properties,[18,19] a comparison study with Igepal was conducted to estimate the magnitude of the Triton penetration enhancement effect. Igepal has been selected by others as a very mild nonionic surfactant with minimal effect on skin permeability.[20]

Solute crystallization tests

The size and morphological characteristics of the test chemicals following solvent deposition onto three substrates were measured by transmitted light microscopy using an Olympus BX50 light microscope equipped with polarizing filters (Olympus Life Sciences, Center Valley, PA). Samples of selected test formulations (10 μL) were pipetted onto glass slides (GS), silanized glass slides (SGS) and HEM. Low doses of solutes (1-3 μg) were deposited on non-absorbing surfaces, higher doses (10-50 μg) were deposited on HEM. Formulations were allowed to evaporate until samples were visually dry. Representative images were captured.

Solvent surface tension and spreading on skin

Surface tensions of the test solvents, solvent mixtures and relevant solute/solvent combinations were measured at room temperature (22-23°C) with a Krüss K100 tensiometer (Matthews, NC) fitted with a Wilhelmy plate. The plate was rinsed and flamed before each measurement. Data presented are an average of ten measurements, with automatic removal of outliers.

A 10 μL aliquot of selected test formulations (IVPT Study 3 below) was deposited onto a split-thickness human skin sample that had been allowed to air dry on a glass slide. Skin was prepared as the in vitro permeation tests. Wheat flour was deposited onto the substrate a few seconds after solvent deposition. The non-adherent flour was removed in order to observe the outline of the solvent spreading area. Sample pictures were taken and the solvent spreading area was analyzed by Image J software.[21] The spreading value was defined as the surface area covered by the solvent relative to that covered by an equal volume of water. Measurements were made for three replicates per formulation obtained from two skin donors.

In vitro skin permeation tests (IVPTs)

Thawed, split thickness skin was sectioned with a scalpel and mounted onto Franz diffusion cells (0.79 cm^2) for the in vitro permeation studies. The donor compartments of the Franz cells had an internal diameter of 10 mm and a height of 9 mm, yielding an aspect ratio (height/radius) of 1.8; the receptor compartment volume was 6 mL. Images of these cells are shown in Gajjar et al.[22] and the donor compartments are highlighted by Tonnis et al.[12] The receptor compartments were filled with Dulbecco's phosphate buffered saline (PBS, pH 7.4) to which 0.02% sodium azide had been added and were magnetically stirred. Assembled cells were placed in aluminum heating/stirring blocks maintained at 37°C in a fume hood as in Gajjar et

al.,[22] resulting in a skin surface temperature of 32 ± 1°C. Skin samples were prescreened using tritiated water as previously described.[23] Samples yielding a water flux greater than 1.6 μL/cm^2 in an hour were discarded. In order to increase the test sensitivity, the skin samples were reordered based on the prescreening results using a randomized complete block design.[23]

Test formulations were applied to the skin in 10 μL aliquots. Solute specific doses were chosen to approximately match those in Hewitt et al.[9] when possible. For niacinamide, the specific dose was chosen as approximately one-half the saturation dose, M_{sat}, calculated as shown in the Appendix. M_{sat} reflects the capacity of the skin to quickly absorb a small topical dose of solute and, in the absence of solute-skin interactions, coincides with the transition between first-order absorption (Dose < M_{sat}) and zero-order absorption (Dose > M_{sat}).[24] Solute doses and solvent compositions tested in this study are shown in Table 2. Four different protocols were examined to test various aspects of the experimental design, as described below.

Study 1. This study was conducted to determine the impact of the extensive skin wash procedure employed in the CE study[9] on skin surface recovery as compared to our standard procedures. Wash procedures were compared for a hydrophilic compound, niacinamide, and a lipophilic compound, testosterone. Our standard procedure (Study 2) is three water washes for hydrophilic compounds and an initial wash with 2% mild hand soap solution followed by three water washes for lipophilic compounds. The CE protocol was an initial wash with 2% mild hand soap followed by seven water washes for all compounds. All washes were collected and counted separately.

Study 2. This study was conducted to test the impact of wetting and non wetting solvents on skin permeation. Donor solutions were prepared with 10 μCi/mL of each of the test permeants. A

volume of 10 μL/cell was pipetted onto the skin. Solutions that did not spread, i.e. the aqueous solutions without surfactant, were carefully dispersed over the skin surface using the pipette tip.

Table 2
Solute doses and solvent compositions tested in this study.[a]

Permeant	M_{sat},[b] μg/cm^2	Dose, μg/cm^2	CE dose,[c] μg/cm^2	Solvents[d]
Study 1 (skin wash protocols)				
Niacinamide	62.4	31.6		water
Testosterone	1.93	8.23	1.64	EtOH (included as a leg of Study 2)
Study 2 (solvent effects)				
Niacinamide	62.4	31.6	-	water, EtOH, 70% EtOH, 2% Triton
Caffeine	15.3	3.16	1.08	water, EtOH, 70% EtOH, 2% Triton
Testosterone	1.93	8.23 & tracer	1.64	EtOH, 70% EtOH
Geraniol	3.17	2.91	2.36; 2.92	H_2O, EtOH, 70% EtOH. 50% EtOH, 30% EtOH, 2% Triton
Study 3 (surfactant effects)				
Caffeine	15.4	3.16	1.08	water; 2%, 0.2% and 0.02% Triton; 2% Igepal
Study 4 (30 min evaporation)				
Geraniol	3.17	2.91	2.36. 2.92	water, EtOH, 50% EtOH

[a] The dose volume was always 10 μL/cell or 12.7 μL/cm^2.
[b] See Appendix for calculation.
[c] Ref. 9
[d] Solvent compositions are % v:v in water; thus 70% EtOH means 70:30 v:v ethanol:water. Surfactant compositions reflect wt% in water of either Triton X-100 or Igepal CA-360.

The receptor solutions were removed at 2, 4, 6 and 24 h post-dose for niacinamide and caffeine; 1, 2, 4, 6 and 24 h for testosterone; and an additional 0.5 h for geraniol, then immediately refilled with fresh buffer. After 24 h, the skin surface was washed with 0.5 mL of Millipore water three times for hydrophilic compounds and four times for lipophilic compounds, with the first wash for the latter employing a 2% mild hand soap solution in water). Washes were pooled. After the skin wash, the cells were disassembled, skin was dissolved in 2 mL of Solvable™ and incubated in an oven at 50℃ overnight. Both chambers were rinsed and included in the skin wash (donor chamber) or 24 h permeation (receptor chamber) for mass balance. All samples were mixed with 5-10 mL of Ultima Gold™ and analyzed by liquid scintillation counting (Beckman LS 6500).

Study 3. This study was conducted to compare the impact of a mildly irritating nonionic surfactant (Triton X-100) on skin permeation compared to a milder nonionic surfactant (Igepal CA-360). Study 3 was similar to Study 2 except that caffeine was the only test solute and several different surfactant compositions including three concentrations of Triton and one of Igepal were examined (Table 2).

Study 4. 30 min geraniol evaporation test. This study was conducted as a diagnostic test for the geraniol leg of Study 2 to determine whether the low recovery of ^{14}C-geraniol dosed from ethanolic solution could be assigned to rapid evaporation following dosing. Procedures were identical to Study 2 except that the study was terminated at 30 min rather than 24 h and a skin wash was not conducted. The wash step was deleted in order to minimize additional loss of geraniol at this time point. Thus the "skin" measurement includes any residual geraniol on the skin surface.

Data analysis

The amount of solute in the receptor solution was determined at each time point and the cumulative permeation was calculated. The amount of solute detected in donor chamber rinses was added to the skin wash; that in the receptor chamber rinses were added to the cumulative permeation at 24 h. Dermal delivery (DD) was defined as the sum of the cumulative permeation at 24 h and amount found in the skin, as in Hewitt et al.[9] DD thus represents the (absorbed + potentially absorbed) dose. SigmaPlot® and Microsoft Excel® software were used for all the statistical analyses. Samples that visually leaked (5/344) were removed from the analysis. Other samples were tested by Outlier Calculator (GraphPad™, Dotmatics) by using the extreme studentized deviate (ESD) method, which is based on a normal distribution and a test statistic (Z),[25] then confirmed by a nonparametric method due to Dixon.[26] Four outliers were detected and

removed by this method. Data were reported as mean ± standard error of the mean. A two-way ANOVA test based on donor and treatment was used to determine the statistical variation. Skin permeation data was analyzed within and between donors, and between the treatment groups. An overall p-value less or equal to 0.05 was considered significant. If significant differences between donors or treatments were detected, post hoc pairwise comparisons were made using the Holm-Sidak method.

Results

Solute crystallization tests

Figure 1 shows crossed polar light micrographs of the four test chemicals deposited on glass slides and silanized glass slides. Small doses (1-3 μg) were sufficient to obtain clear crystalline images on these non absorbing surfaces, except for niacinamide on untreated glass, which tended to spread widely at doses less than 10 μg. Niacinamide and caffeine formed highly crystalline aggregates on both substrates. These two solutes tended to concentrate at the perimeter in patterns that were usually irregular, but occasionally highly symmetrical (see caffeine on silanized glass). Different morphologies were observed on untreated and silanized glass. Irregular aggregates of needle-like crystals were observed for testosterone on both substrates. For geraniol, there was no evidence of crystallization on either substrate. This is not surprising since geraniol is a liquid at room temperature. Thus it serves as a negative control.

Figure 2 shows crossed polar light micrographs of the four test solutes deposited onto HEM. Higher doses were required to observe crystals on this absorbing substrate. There was always an irregular background level of transmitted light intensity, presumably associated with

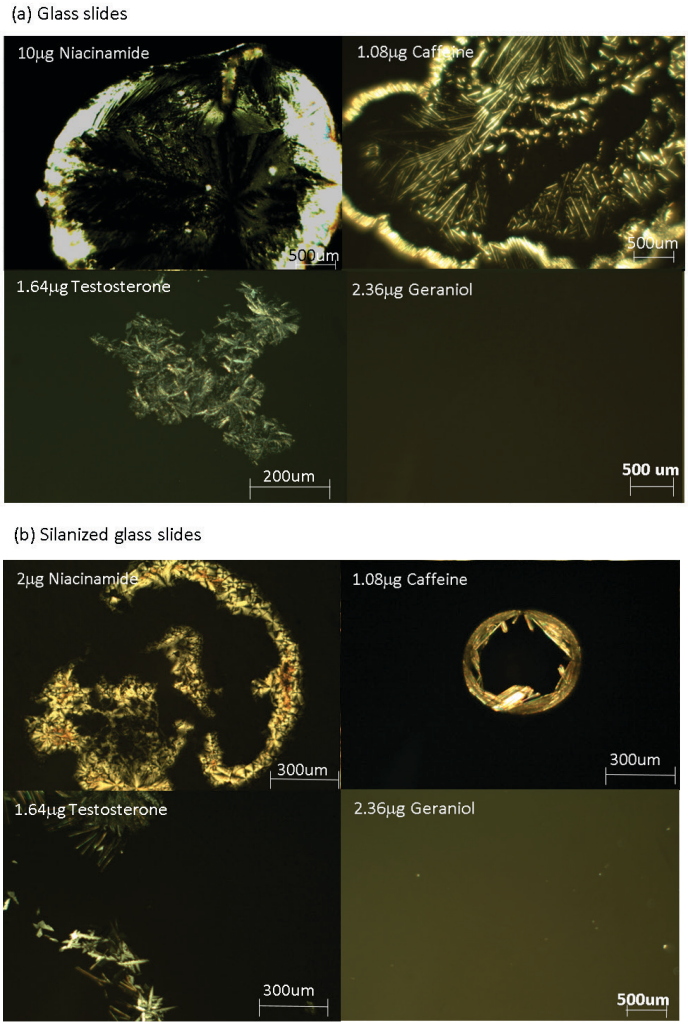

Figure 1. Light micrographs of test solutes dissolved in 50% EtOH (50:50 v:v EtOH:water) deposited in 10 µL aliquots onto (a) glass slides and (b) silanized glass slides. Dose and solute are indicated on each panel. Images were captured by transmitted light under cross-polarized filters.

tissue components. Crystallization could clearly be detected for both niacinamide and caffeine, with the order of intensity (visual estimate) being water > EtOH >> 70% EtOH ~ 2% Triton. These estimates were made by qualitatively comparing the brightness of the angular, crystalline deposits with the more rounded, diffuse patterns present in all of the samples. The latter patterns cannot be seen in the images with highest crystallinity (niacinamide and caffeine in water), as the incident light intensity was reduced so as to not overexpose the crystalline deposits. There was some evidence of crystallization for testosterone dosed in EtOH, but little or none when dosed

from 70% EtOH. The sample dosed with an aqueous solution of geraniol showed no evidence of crystallization, as expected.

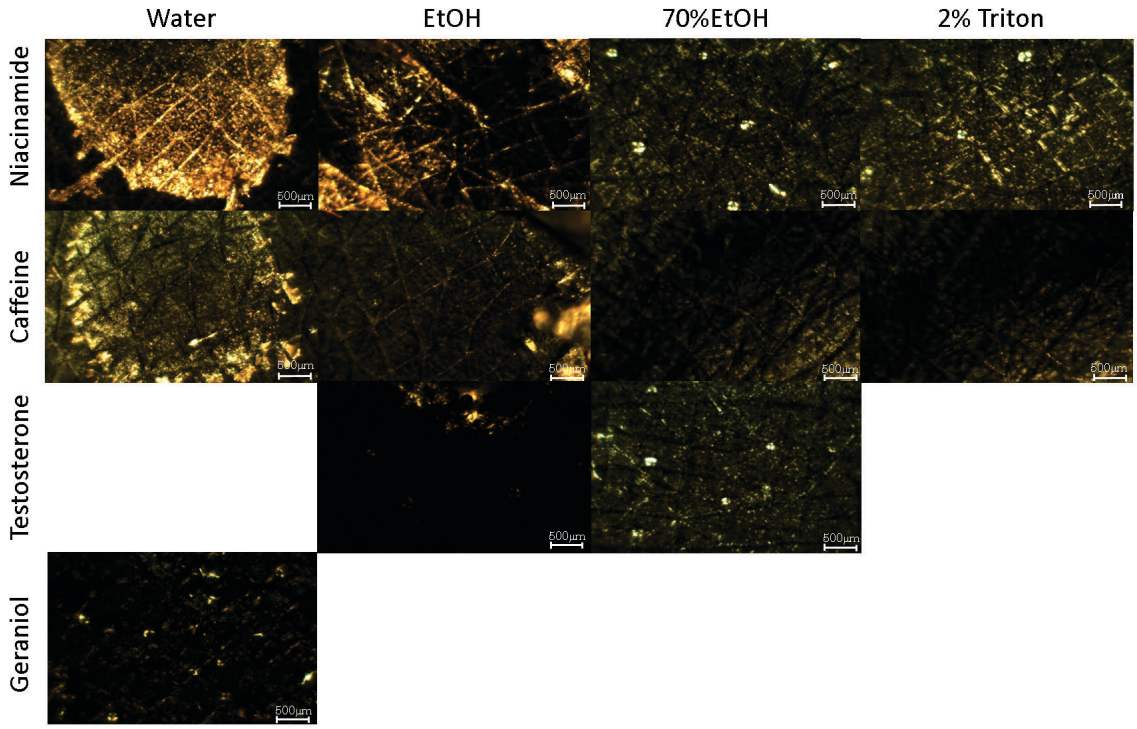

Figure 2. Light micrographs of test solutes deposited in 10 µL aliquots onto human epidermal membrane (HEM) from four solvents. Doses were 50 µg for niacinamide, 10 µg for caffeine, 30 µg for testosterone and 50 µg for geraniol. Images were captured by transmitted light under cross-polarized filters.

Solvent surface tension and spreading on skin

Surface tensions (SFT) of the test solvents and selected solute/solvent combinations measured at room temperature are shown in Table 3. Selected literature values at 25°C are shown for comparison. The SFT values for water (72.4 mN/m) and the ethanol:water mixtures were comparable to literature values. SFT for ethanol, all ethanol:water mixtures and all surfactant compositions tested was in the range 22-36 mN/m. Niacinamide and caffeine, tested in water at concentrations comparable to those in the IVPT studies, had little impact on aqueous SFT, whereas geraniol lowered SFT to 49.8 mN/m. Thus, geraniol has an appreciable surface excess in aqueous solutions as might be expected for a weak amphiphile.

Table 3
Surface tension of test solvents and solutions at room temperature

ID	Avg. SFT, mN/m
Test solvents[a]	
Water	72.4, 72.4[b]
30% EtOH	35.8[b]
50% EtOH	29.3, 29.5[b]
70% EtOH	26.6, 25.9[b]
Ethanol (EtOH)	22.0[b,c]
2% Igepal CA-360	32.2
2% Triton X-100	31.7
0.2% Triton X-100	31.1
0.02% Triton X-100	30.1
Test solutes in water	
2.5 g/L niacinamide (25 μg/10 μL)	67.7
0.25 g/L caffeine (2.5 μg/10 μL)	69.1
0.23 g/L geraniol (2.3 μg/10 μL)	49.8

[a]Balance of each solution is water
[b]Interpolated value at 22.5°C from Ref. [27]
[c]Ref. [28]

Spreading capability of geraniol dose solutions applied to HEM as measured in the "wheat flour" protocol is summarized in Table 4. All ethanol:water mixtures as well as 2% Triton X-100 led to spreading areas at least twice that of water. Ethanol spread rapidly to cover an area approximately four times that of water.

Table 4
Spreading ratio of test formulations from IVPT Study 3 on excised human skin[a]

Formulation	Mean ± SD (n = 3)
Water	1.00[b]
30% EtOH	2.10 ± 0.57
50% EtOH	2.44 ± 0.55
70% EtOH	2.99 ± 0.58
EtOH	4.18 ± 1.37
2% Triton	3.06 ± 0.68

[a]All formulations contained 0.23 g/L geraniol (2.3 μg/10 μL) in addition to ingredients listed. A separate study (data not shown) confirmed that the presence of geraniol in the water formulation did not measurably increase spreading versus water alone.
[b]Water containing 0.23 g/L geraniol served as the baseline in each study. The absolute spreading area for the water treatment (10 μL) was 0.33 ± 0.04 cm^2 (n = 3).

Skin wash procedure (IVPT Study 1)

This study tested the impact of extra skin washes on the recovery of niacinamide and testosterone from the skin surface following a 24 h IVPT study. The results are presented in Fig. 3. No significant differences between the two wash methods in permeation, skin concentrations or total skin wash were observed for either compound. The majority of the skin surface recovery was found in the first two washes for niacinamide and in the first 3-4 washes for testosterone. Therefore, for subsequent experiments, the three water wash procedure was used for hydrophilic compounds and the four wash procedure (the first with mild hand soap) was used for lipophilic compounds.

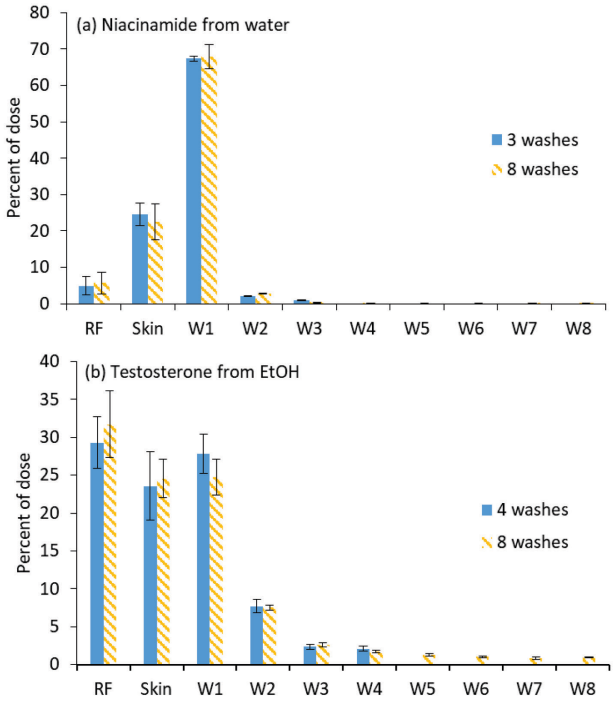

Figure 3. Distribution of radioactivity associated with (a) ^{14}C-niacinamide and (b) ^{14}C-testosterone in receptor fluid, skin and sequential skin washes under two skin wash protocols. Values represent the mean ± SD of three donors with 4-6 replicates per donor (n = 14-16 total).

Solvent effects on finite dose absorption (IVPT Study 2)

The time course of permeation of the hydrophilic test compounds through human skin from four solvent systems is shown in Fig. 4 and the associated mass balance at the end of the

study is shown in Fig. 5. Niacinamide (Figs. 4a, 5a) was slowly absorbed, with less than 4% of the dose permeating in the first 6 h and 5-21% permeating in 24 h. The order of permeation was 70% EtOH ~ 2% Triton > EtOH > water, with a more than four-fold difference between 70% EtOH and water at 24 h. Peak fluxes of 0.27-1.0% of dose/h (0.085-0.32 $\mu g \cdot cm^{-2} h^{-1}$) were achieved over the range 6-24 h post-dose. We noted that the water solution tended to reform small droplets on the skin surface after being dispersed with the pipet tip. For 70% EtOH, 21% of the radiolabel was found in the receptor fluid (RF) another 50% was found in the skin, leading to a dermal delivery (DD) equal to 71% of the dose. For water, 67% of the radiolabel was found in the wash and 27% in the skin; thus, DD was only 32%. The other two treatments led to

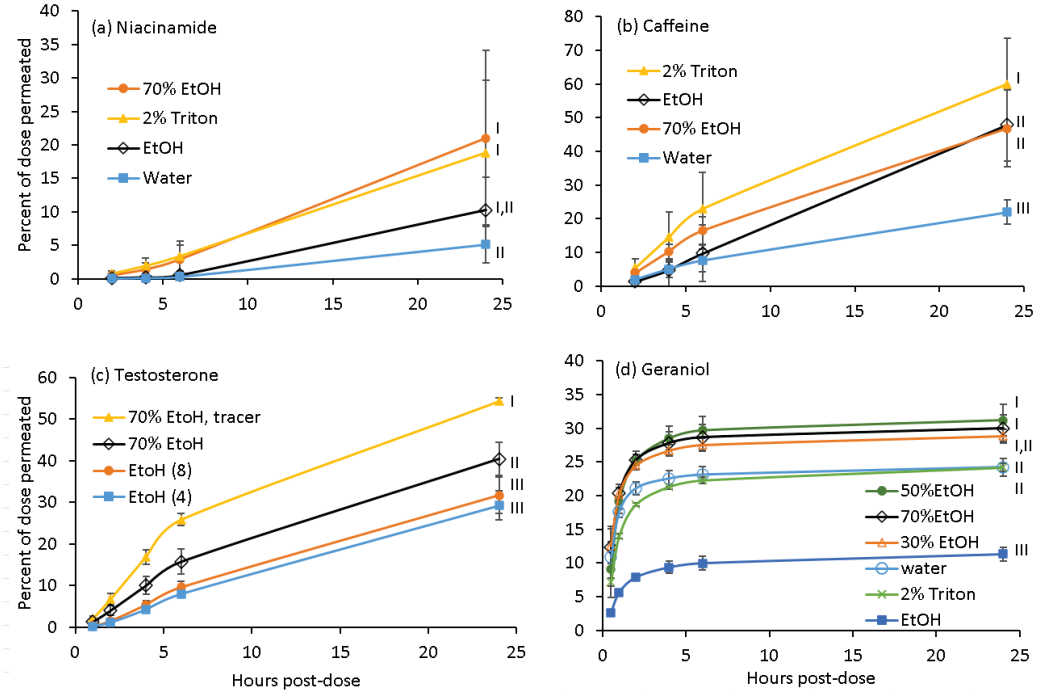

Figure 4. Permeation time course of radioactivity associated with (a) 31.6 $\mu g/cm^2$ niacinamide; (b) 3.16 $\mu g/cm^2$ caffeine; (c) 8.23 $\mu g/cm^2$ & tracer level testosterone; and (d) 2.91 $\mu g/cm^2$ geraniol through excised human skin following application from different solvents. Values represent the mean ± SD of three donors, with 4-6 replicates per donor (n = 14–16 total). Treatments sharing the same Roman numeral are not significantly different at 24 h post-dose.

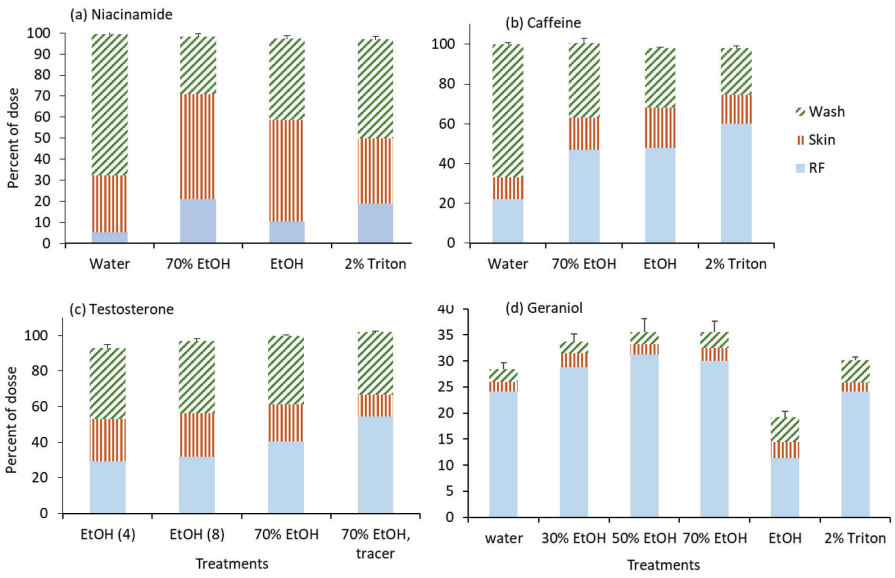

Figure 5. Distribution of radioactivity in wash, skin and receptor fluid at 24 h post-dose for the IVPT studies shown in Fig. 4.

intermediate values. Inter-donor variability was also extremely high, with a relative standard error (RSE) of 58%. This confirms results seen an earlier solvent-deposition study with niacinamide.[13]

Results for caffeine (Figs. 4b, 5b) were qualitatively similar to those for niacinamide, although caffeine is a better skin permeant. Permeation after 6 h ranged from 7-23% and at 24 h from 22-60%. Fairly constant peak fluxes of 1.4-4.4% of dose/h (0.044-0.14 $\mu g \cdot cm^{-2} h^{-1}$) were achieved over the range 2-6 h post-dose. The order of permeation was 2% Triton > 70% EtOH ~ EtOH > water, with a 2.7-fold difference between 2% Triton and water at 24 h. The water solution beaded up after dispersal with the pipet tip in a manner similar to niacinamide. DD for the Triton solution was 75%, whereas for water it was only 33% with the remaining 67% in the skin wash. The other two treatments yielded intermediate values. The inter-donor variability (RSE 22%) was less than that of niacinamide, leading to a statistical breakout of the 24 h permeation results into three distinct groups. Cumulative permeation of caffeine from water at 24 h (22%) was less than that reported by Hewitt et al. from PBS (39%).[9]

For testosterone (Figs. 4c, 5c), permeation at 6 h ranged from 8-26% of the dose and at 24 h from 29-54% of the dose. Fairly constant peak fluxes of 1.7-2.9% of dose/h (0.14-0.24 $\mu g \cdot cm^{-2} h^{-1}$) were achieved over the range 2-6 h post-dose. The tracer dose had a higher peak flux when expressed in % of dose/h (4.8%), but a negligible chemical flux. The order of permeation was 70% EtOH (tracer) > 70% EtOH > EtOH. Permeation of tracer level testosterone was significantly higher than that of 8.2 $\mu g/cm^2$ testosterone, as might be expected given the saturation dose (M_{sat}) of 1.93 $\mu g/cm^2$ estimated in Table 2, and both 70% EtOH treatments yielded significantly higher permeation than the two EtOH treatments. Inter-donor variability of 24 h permeation (RSE) was only 6.7%, much smaller than that for niacinamide and caffeine. All of the 24 h permeation results reported here were substantially higher than the mean value of 3.4% reported by Hewitt et al. for 1.64 $\mu g/cm^2$ testosterone in EtOH.

Geraniol (Figs. 4d, 5d) was absorbed much more rapidly than the other test compounds, with no evidence of a diffusion time lag. Peak fluxes with values of 22-25% of dose/h (0.64-0.73 $\mu g \cdot cm^{-2} h^{-1}$) were achieved within 30 min post-dose for three of the test formulations, whereas the other three peaked at 6-20% of dose/h in the 30-60 min timeframe. Expressed as % of dose/h, the higher flux values are 5- to 6-fold higher than those for caffeine and testosterone and more than 20-fold higher than those for niacinamide. Geraniol also evaporated rapidly as evidenced by the low recovery of radioactivity at the end of the study (19-34%) and the attainment of a near plateau in the permeation plots within 4 h post-dose. The order of permeation was 50% EtOH ~ 70% EtOH ~ 30% EtOH > water ~ 2% Triton >> EtOH, with a 2.8-fold difference between 50% EtOH and EtOH. Note that 2% Triton in water did not increase permeation relative to water alone. EtOH yielded the lowest permeation (11%) and also the lowest total recovery (19%). Inter-donor variability RSE of 24 h permeation was 5.1%, slightly less than that for testosterone

and much less than the hydrophilic compounds. In comparison with the Hewitt et al. study,[9] 24 h permeation from water was slightly lower in the present study (24% vs. 32%), whereas permeation from EtOH was substantially higher (11% vs. 4%).

Following completion of the solvent effects studies, two diagnostic studies were conducted to further examine specific aspects of the study design. Results are presented in the next two sections.

Surfactant effects on absorption (IVPT Study 3)

This study examined the impact of Triton X-100 on skin permeation of caffeine as a function of concentration and also in relation to a nonionic surfactant, Igepal CA-360, considered by others to be mild.[29] Three Triton concentrations and 2% Igepal were tested versus a water control. Results are shown in Fig. 6.

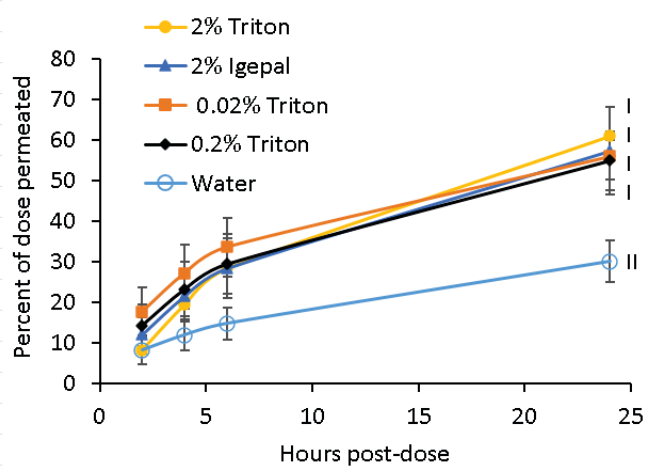

Figure 6. Permeation time course of radioactivity associated with 3.16 µg/cm^2 caffeine following application from water and surfactant/water mixtures. Values represent the mean of 4-5 replicates from one skin donor. Treatments sharing the same Roman numeral are not significantly different at 24 h post-dose.

Cumulative permeation of caffeine at 24 h was comparable for all four surfactant treatments and was about twice that from water. Permeation from 0-6 h was directionally higher for the 0.02% Triton treatment than for Igepal or the higher Triton concentrations. All surfactant treatments had SFTs in the range 30-32 mN/m, whereas the water solution had an SFT of 69

mN/m (Table 3). Spreading ratios versus water for closely related geraniol solutions on excised skin ranged from 2.1-4.2 (Table 4). Given the lack of a dose-response to Triton and the equivalence of the Triton and Igepal results, we attribute the higher delivery of caffeine from the surfactant formulations to improved deposition and spreading on skin rather than an impact on skin permeability.

30 min geraniol evaporation test (IVPT Study 4)

This study probed the substantial loss of radioactivity in the geraniol IVPT study shown in Figs. 4d and 5d. The study was conducted similarly to the solvent effects study except that only three formulations were tested, the test was terminated after 30 min, and no skin wash was conducted. Results are shown in Fig. 7. Total recovery of geraniol ranged from 24-45% with most of the radioactivity found in the skin. The EtOH solution led to the lowest mass balance and the lowest permeation into the receptor solution. Comparison with Fig. 5 suggests that most of the geraniol evaporation in the solvent effects study occurred in the first 30 min of the test.

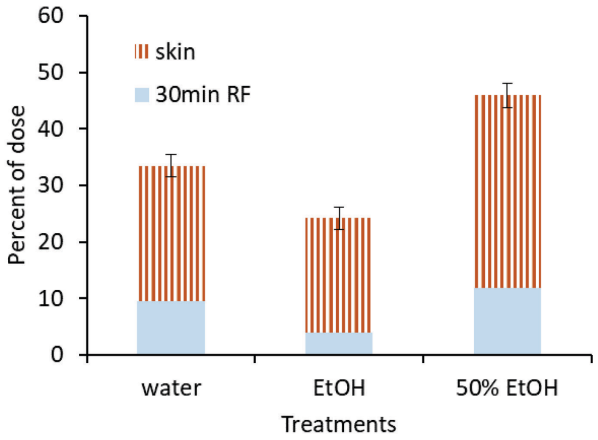

Figure 7. Distribution of radioactivity associated with ^{14}C-geraniol 30 min following dosing on human skin from three solvents. Values are the mean of two donors with two and four replicates, respectively.

Discussion

Skin surface energy and wettability has been extensively studied,[4] as has the impact of drug crystallization on skin permeation.[1] Evaporation of volatile solvents from the skin has also received attention[22,30] and has been quantitatively treated for single solvent systems.[3,24,31] It is evident that all three of these factors come into play when solutes are solvent-deposited on skin. The initial spreading rate, evaporation rate and (most likely) convective transport rate into the upper stratum corneum[24] determine the initial distribution of the solute on and in the skin. Deposited solute can then precipitate or remain dissolved, depending on its location and solubility in the tissue components. Precipitation can involve both aggregation and different crystal polymorphs/habits and is not likely to be uniform, cf. Figs. 1 and 2. We discuss below each of these factors and how they may impact percutaneous absorption. In this discussion the term "deposition" refers to the initial application and spreading of the formulation on the skin, including sorption of mobile components into the upper few layers of the SC (the so-called "deposition layer").[24] "Penetration" refers to both the sorption process and to further transport of these components into deeper layers of the SC or skin. "Permeation" indicates the penetration of a component all the way through the skin and into the receptor fluid (in vitro) or the systemic circulation (in vivo), whereas "skin permeability" refers to the quality of the skin barrier function. Thus a "deposition aid" can increase skin penetration or permeation without increasing skin permeability. A "penetration enhancer", on the other hand, can increase these quantities by either improving deposition or by increasing skin permeability. A composition that disrupts the skin barrier at high doses, e.g. an ethanol:water mixture, may function simply as a deposition aid at low doses, as is argued below.

Solvent wetting and spreading on skin

We adopt here a simplified model for skin surface energy in which it is considered to be an apolar surface with a surface free energy γ_{sv} dominated by the Lifshitz-van der Waals component γ^{LW}.[4] This approximation is reasonable for excised skin from a skin bank, which has been thoroughly washed and disinfected prior to storage, removing most or all of the sebum layer. In our experience, further treatment, e.g. by heat separation to remove the dermis, does not alter skin surface energy or solvent spreading on skin. The work of spreading wetting per unit area is[32]

$$W_S \equiv \sigma_{lsv} = \gamma_{sv} - \gamma_{lv} - \gamma_{ls} \quad (1)$$

where σ_{lsv} is the spreading tension, γ_{ls} is the liquid/solid interfacial tension and γ_{lv} is the liquid surface tension. A liquid will spread if $W_S \geq 0$. These values are related to the contact angle θ by Young's equation,

$$\gamma_{sv} = \gamma_{ls} + \gamma_{lv} \cos\theta \quad (2)$$

where the film pressure π_e has been neglected because skin is a low energy surface.[4] Inserting Eq. 2 into Eq. 1 and simplifying yields

$$W_S = \gamma_{lv}(\cos\theta - 1) \quad (3)$$

Thus a liquid will spread spontaneously on a smooth surface only if θ approaches zero. Surface roughness complicates the picture, but does not change these fundamental relationships.[4] Minimizing γ_{lv} and γ_{ls} by, e.g., adding a surfactant to the liquid phase that adsorbs at both the liquid and solid interfaces increases W_S and cos θ, driving θ toward zero.

The surface free energy of sebum-free skin, γ_{sv}, was determined by Mavon and coworkers to be in the range 31-32 mN/m.[4] The related critical surface tension, γ_c (the maximum surface tension required to fully wet the skin), has been measured to be in the range 22-30 mN/m for

both solvent-treated and untreated skin.[33] Schott hypothesized that furrows and air bubbles rather than very low surface energies lead to the low values of γ_c. The data in Table 3 show that all of the organic solvent and surfactant/water combinations examined in the present study lower the initial value of γ_{lv} to a value comparable to γ_{sv} and approaching γ_c for sebum-free skin. For the EtOH:water formulations, γ_{lv} will clearly increase with time due to the more rapid evaporation of ethanol; however, the data in Table 4 show that significant spreading occurs prior to this increase in γ_{lv}. This factor favors improved absorption.

We hypothesize that there is an osmotic component to the improved dermal delivery of all solutes from EtOH:water compositions versus EtOH alone. All solvents placed in contact with air-dried skin experience a driving force for penetration comprised of a Fickian (diffusive) component and also a convective component due to capillary action. For purely ethanolic solutions the convective flow is opposed by an osmotic gradient driving water from the skin to the applied formulation. This may negate the convective flow of ethanol. The more rapid evaporation of pure ethanol may also contribute to lowered delivery. The difference can be dramatic – see the permeation profile for geraniol (Fig. 4d). Even larger differences have been observed by others for this same solute – see Figs. 1G and 1H in Hewitt et al.[9] These differences cannot be explained on a spreading basis (Table 4) or a crystallization basis since geraniol is a liquid at skin temperature. Net, there is considerable evidence that EtOH:water solutions applied in small quantities improve dermal delivery without impacting skin permeability. It may be inferred that the same applies to hydroalcoholic gels comprised of isopropanol, water, and a polymeric thickener.

Solute precipitation on the skin

It is well known that solute precipitation within the dose form or on the skin surface can retard the absorption of both topical and transdermal drugs.[1] Less well understood is the possible impact of precipitation on solute doses from volatile solvents that approach the capacity of the skin to dissolve them. Furthermore, the line between solvent deposition effects and excipient effects on skin permeability is a blurry one. It depends strongly on skin load and duration of the exposure, as do other aspects of dermal absorption.[3]

When deposited at low doses on non absorbing surfaces, all the test solutes except geraniol formed highly crystalline deposits (Fig. 1). Higher doses that matched or sometimes exceeded the estimated dose required to saturate the upper layers of the stratum corneum (M_{sat}, Table 2) were required in order to detect crystallization following deposition on skin (Fig. 2). For the hydrophilic compounds the order of crystallinity, as assessed by transmitted light intensity, was water > EtOH >> 70% EtOH ~ 2% Triton. The extent of spreading, as inferred from closely related geraniol solutions (Table 4), was EtOH > 2% Triton ~ 70% EtOH >> water. These patterns do not have a simple relationship. In particular, it is not an inverse relationship in which greater spreading leads to lower crystallinity. The ethanolic solutions spread very rapidly, but left more solute deposited on the skin surface as discussed in the preceding section. However, the order of crystallinity *does* correspond inversely to the order of skin permeation, especially if both the 6 h and 24 h permeation values are considered (Fig. 4). Testosterone (30 μg, Fig. 2) showed some evidence for crystalline deposits from EtOH, but none from 70% EtOH. The bright areas appearing in the 70% EtOH micrographs for testosterone and niacinamide have a rounded appearance similar to those for geraniol in water. We attribute this birefringence to skin components, possibly SC lamellar lipids (note the similarity of some bright spots to the Maltese

crosses characteristic of lamellar liquid crystals).[34] Net, both spreading on the skin surface and penetration of the solvent into the skin following spreading play a role in the eventual absorption of deposited solute.

Solvent evaporation and potential impact of solvents and surfactants on skin permeability

Evaporation of water and ethanol from either skin or glass substrates mounted in the Franz diffusion cells employed in the present study has been previously studied in our laboratory.[22] Ethanol evaporated 4-5 times faster than water in either a bench top or fume hood environment. The difference may be attributed to a 2.4-fold higher vapor pressure and a 2.7-fold lower heat of vaporization of ethanol (per gram) at 32°C, the latter leading to lower evaporative cooling of the surface. Furthermore, ethanol spreads more widely (Table 4), leading to a larger surface area for evaporation. Evaporation of ethanol:water mixtures has been studied from droplets.[35] Although the details are complex, it is safe to say that evaporation rates are intermediate between pure ethanol and pure water, with the ethanol component evaporating more rapidly than water.

Using the evaporation rates measured in a very similar environment by Gajjar et al.[22] and the solvent dose applied in the present study (10 μL/0.79 cm^2), we can estimate that ethanol evaporated from the skin surface in about 2 min and water (if evenly distributed) evaporated in 9-10 min. Because both solvents also penetrate into the skin and can diffuse back to the skin surface, slow evaporation continues for a longer period. Based on evaporative water loss measurements from human skin in vivo summarized by Saadatmand and coworkers[36] we can estimate that evaporation of excess water from the skin was essentially complete by 20 min post-dose. This estimate could be raised by as much as a factor of 2.5 by the fact that the water solutions did not fully spread on the skin surface even after dispersion with a pipette tip, as noted in Table 4 and the IVPT methodology section. This argument does not apply to ethanolic

solutions or ethanol:water mixtures, which spread very rapidly and evenly on the skin. In any case, solvent evaporation was complete well before appreciable accumulation of solute in the receptor fluid was achieved.

Permeation of ethanol through human skin following a 10 μL dose under conditions very similar to the present study[30] was about 0.22% of the dose after 24 h and was 97% complete after 4 h. This corresponds to a total permeation of 17 $\mu g \cdot cm^{-2}$ over 24 h. The flux peaked at about 30 $\mu g \cdot cm^{-2} h^{-1}$ between 10 and 20 min post-dose. Applying these results to the present study, we conclude that for most of the 24 h exposure period, there was very little ethanol in the skin. Furthermore, formulations containing less ethanol, i.e. the EtOH:water mixtures, yielded higher permeation than pure ethanol. Although it is well known that the sustained presence of ethanol in higher concentrations will swell the SC and increase skin permeability,[37,38] it is hard to argue that ethanol is acting to disrupt SC lipids under the present, small dose conditions. Based on the micrographs in Fig. 2 and the permeation results in Fig. 4, it is much more likely that the combination of ethanol and water serves as a skin deposition aid. This conclusion is supported by other studies employing ethanol under finite dose conditions. Oliveira et al.[2] studied the impact of added EtOH on the human skin permeation of methyl paraben from saturated solutions in isopropyl myristate (IPM), dimethyl isosorbide (DMI) and Transcutol™ (TC) under finite dose conditions similar to present studies. They concluded that "The presence of EtOH in the IPM, DMI and TC formulations tested had little effect on the flux of methyl paraben through the skin." They furthermore measured ethanol evaporation from the skin under their test conditions and found that "most of the volatile solvent evaporated ~6 min after application." Intarakumhaeng et al.[6] evaluated finite dose delivery of tracer amounts of three hydrophilic solutes and three lipophilic solutes from water, ethanol, and two other solvents (butanol and propylene glycol). In

only one of these studies (corticosterone) did ethanol provide even directionally improved delivery versus the other four solvents. We conclude that there is little evidence for skin permeability enhancement by ethanol when applied to unoccluded skin in small volumes.

We now turn to the MD simulations of Gupta et al.[8] described in the Introduction that suggest that even modest concentrations of ethanol in water can extract free fatty acids and (at higher concentrations) ceramides from the SC. Taken at face value, these calculations imply that ethanol damages the SC in a dose-dependent manner. However, human experience with ethanol:water compositions applied to skin is quite different. Aqueous solutions containing high volume fractions of ethanol are well tolerated on skin and were the basis of several membrane-reservoir transdermal systems developed by Alza and Ciba-Geigy in the 1970s and '80s.[37,38] In their work with ternary systems containing also nitroglycerin, Berner and coworkers found that nitroglycerin flux and ethanol flux increased proportionally with increasing ethanol volume fraction (f_e) for $f_e < 0.7$ (corresponding to mole fraction ethanol $x < 0.42$), then both fell off for $f_e > 0.7$.[38] The authors state "The increased nitroglycerin flux from ethanol-containing donors reverts to its standard value of 10 μg/cm^2/h when the ethanol donor is removed…demonstrating rapid and complete reversibility." Scheuplein found that pretreatment of isolated human SC with ethanol had a milder (possibly insignificant) effect on its permeability to tritiated water, in contrast to acetone, ether and chloroform-methanol.[39] (p. 1748) The present study (Figs. 4d, 5d) shows the impact of ethanol on geraniol permeation from ethanol:water mixtures peaks at 50% v:v ethanol; larger amounts of ethanol actually *decrease* permeation. Gupta et al. themselves state that the use of ethanol in household products is safe, citing two 2007 studies of hand disinfectants.[8] Net, both in vitro and in vivo human studies show that infrequent, small exposures to ethanol are non damaging to skin. Repeated exposures to alcohol

hand rubs are quite another matter, where both ethanol and the milder isopropanol dry the skin and can lead to stinging and chronic contact dermatitis in occupations requiring frequent skin disinfection.[40]

Several further aspects of current MD simulations warrant discussion. Most of these including Gupta's[8] represent a single lipid bilayer comprised of three representative lipids – a fatty acid, a ceramide and cholesterol – in equimolar proportions. The bilayer exists in an essentially infinite aqueous environment that may contain additional solutes such as ethanol. Lipid representations may be molecular or coarse-grained. Such representations lack many features of SC structure and experimental conditions that, in our view, are essential to understanding both ethanol effects on SC lipids and finite dose exposures in skin. These include complex lipid mixtures,[41] multi-bilayer (or even more complex) configurations,[42-44] a cornified cell envelope,[45] stress and strain related to co-swelling of corneocytes by the ethanol:water mixture,[37] and (importantly!) wetting and spreading of the formulations on skin as discussed in this report. Consequently MD simulations serve as a complementary tool to traditional methods such as spectroscopy and macroscopic observations for elucidating transport mechanisms, but they do not replace them. Nor are they inherently more accurate. They are simply one more tool in the tool box.

A similar deposition aid argument may be developed for the surfactant treatments in this study. Exposure to Triton X-100 is damaging to reconstructed human epidermis[19] and the substance is classified as a Class 2 skin and eye irritant by the European Chemicals Agency.[46] Melot et al.[47] characterized it as a "lipid-extracting" permeation enhancer, evidently following Karande,[48] but showed only that it slightly increased the concentration of trans-retinol in the upper layers of the stratum corneum following topical application on human volunteers from an

oil-based formulation. At a 2% concentration it increased skin permeation versus water alone for the solutes examined in this study (Fig. 4). But there is no evidence of a dose-response. Triton concentrations of 0.2% and 0.02% were equally effective as 2% Triton in promoting skin permeation (Fig. 6). Moreover, the Triton solutions and a 2% solution of Igepal CA-360 were equally effective. Igepal was chosen by German toxicologists as a particularly mild nonionic surfactant to use as a solubilizer for testing the barrier function of reconstructed human skin models.[20] The property shared by all of these surfactant solutions is their ability to lower the surface tension of aqueous solutions to the range 30-32 mN/m (Table 3), a value that ensures rapid wetting and spreadability on skin. Consequently, under the limited exposure conditions of the present study, it is more likely that Triton and Igepal act as skin deposition aids, not skin lipid disruptors.

The low delivery of geraniol through the skin from ethanol relative to water (Fig. 4d) and especially EtOH:water mixtures was of particular interest. Hewitt et al.[9] observed a similar or even larger difference for three of four compounds tested in ethanol and 0.01 M PBS buffer. Since EtOH and EtOH:water mixtures spread rapidly on skin whereas water does not, the poor delivery cannot be attributed simply to wetting or spreading. More geraniol evaporated from the EtOH treatment than from either water or EtOH:water mixtures (Fig. 5d), and this evidently happened very rapidly (Fig. 7). The likely cause is the rapid evaporation of ethanol or a lower rate of convective transport of the dose solution into the upper SC, or a combination of both. The practical implications of this finding are that neither water, PBS buffer nor ethanol are optimum vehicles for topical delivery of drugs or solvent deposition studies for test chemicals. Their selection may lead to an underestimation of dermal delivery relative to mild topical products that simply spread more uniformly on the skin. The choice of PBS buffer and/or ethanol for the IVPT

studies reported by Hewitt et al.[9] very likely contributed to the large amounts of many test chemicals found in the skin wash at 24 h post-dose.

Conclusions and recommendations

A combination of studies including spreading and crystallization on skin, in vitro skin permeation and associated evaporative loss of four test compounds applied to human skin from simple solvent systems was conducted to examine the various factors impacting topical delivery from finite doses of volatile solvents. Spreading, crystallization, solvent evaporation rate and (possibly) convective transport rate into the skin were all found to play a role. Ethanol and water, taken alone, were less-than-optimum solvents. Much better delivery was obtained with EtOH:water mixtures and dilute solutions of nonionic surfactants, with no evidence of skin barrier disruption under the test conditions employed. We recommend the use of aqueous ethanol and/or dilute nonionic surfactant solutions, applied at a low dose under unoccluded conditions, for testing the skin permeation properties of test chemicals under finite dose conditions. The use of water or ethanol alone, or related high surface energy solutions like phosphate buffered saline, may lead to underestimation of dermal delivery and/or percutaneous absorption of both hydrophilic and lipophilic solutes relative to properly formulated products. Although not examined in this study, we expect that aqueous isopropanol solutions, with or without gelling agents, will give comparable results to aqueous ethanol. The latter compositions are commonly employed as the basis for gel formulations of topical drugs, in part for the reasons demonstrated in this investigation.

It is likely that more advanced imaging techniques including confocal fluorescence and confocal Raman spectroscopy will yield more detailed information than polarized light microscopy regarding solids deposited on and near the skin surface. We recommend further

investigation into the use of these techniques to better understand crystallization and re-dissolution processes associated with solvent deposition of dissolved solutes on the skin.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgements

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Supplementary material

Tabular data for the IVPT studies reported in Figures 3-7 and a description of the log linear cosolvency approximation may be found in the Supplementary Material associated with this article.

Appendix – Calculation of the saturation dose, M_{sat}

M_{sat} and related quantities, e.g. Kissel's NDERM[49] or variations thereof,[3] attempt to identify the crossover point at which a finite dose begins to behave as an infinite dose, or vice-versa. For the case of partially hydrated skin, M_{sat} was originally defined by Kasting and Miller as follows:[24]

$$M_{sat} = h_{dep} C_{sat} = 0.1 h_{sc} \widetilde{K}_{sc/w} S_w \quad \text{(A1)}$$

where h_{sc} = 13.4 μm is the SC thickness, S_w is solute water solubility at 32°C, and

$$\widetilde{K}_{sc/w} = 0.040 K_{oct}^{0.81} + 4.057 K_{oct}^{0.27} + 0.359 \quad \text{(A2)}$$

is the solute SC/water partition coefficient in partially hydrated skin. Eq. A2 was subsequently derived and explained by Nitsche et al.[50] The term h_{dep} in Eq. A1 represents the deposition layer, taken by Kasting and Miller to be 10% of the SC thickness. It corresponds closely to the stratum disjunctum identified by light microscopy, which is generally considered to be more permeable than the lower layers of the SC.[51] The three terms on the right-hand side of Eq. A2 reflect solute partitioning into SC lipids, binding to SC keratin and partitioning into water associated with the SC corneocytes, respectively.

Equations 1 and 2 work well for poorly soluble compounds having a constant $\widetilde{K}_{sc/w}$, but they fail for highly soluble solutes for which $\widetilde{K}_{sc/w}$ varies with concentration. These include solutes like niacinamide that self-associate in aqueous solution[52,53] or solvents like ethanol that swell the skin in combination with water.[37] This is a known limitation of the model. Dancik et al.[54] corrected for this by limiting C_{sat} to the density ρ of the solute, an approximation we have used until recently. However, Tonnis et al.[12] have now found that better results with several highly soluble compounds were obtained by limiting C_{sat} to $\rho / 3$. This is the approximation

employed to estimate the M_{sat} values in Table 2. Niacinamide was the only solute impacted by these solubility constraints. The M_{sat} calculation for niacinamide was thus:

1. Choose octanol/water partition coefficient appropriate for saturated solutions.
    a. log K_{oct} (dilute) = –0.37; K_{oct} = 0.427
    b. log K_{oct} (sat) = –1.39 ; K_{oct} = 0.0407 selected (see Table 1)
2. Calculate $\widetilde{K}_{sc/w}$ from Eq. 2.

    $\widetilde{K}_{sc/w}$ = 0.040 (0.0407)$^{0.81}$ + 4.057 (0.0407)$^{0.27}$ + 0.359

    = 0.0030 + 1.710 + 0.359

    = 2.072
3. Calculate C_{sat} from Eq. 1.

    C_{sat} = $\widetilde{K}_{sc/w}S_w$ = 2.072 × 534 g/L × 1 L/1000 cm^3

    = 1.106 g/cm^3
4. Compare C_{sat} with ρ / 3. Choose the smaller of the two values. The density of neat niacinamide is 1.4 g/cm^3.[55]

    C_{sat} = min (1.106, 1.4/3) g/cm^3 = 0.467 g/cm^3
5. Calculate M_{sat} from Eq. 1.

    M_{sat} = $0.1h_{sc}C_{sat}$ = 0.1 (0.00134 cm)·(0.467 g/cm ^{3})

    = 62.6 × 10^{-6} g/cm^2 = 62.6 μg/cm^2

This algorithm is easily programmed. As pointed out by Tonnis et al.,[11] the ρ / 3 limitation finds some support from skin swelling studies with ethanol:water mixtures conducted by Berner et al.[37] It is an artifice for allowing "swelling-like" phenomena to occur without explicitly changing the dimensions of the SC. This saves considerable numerical complexity.

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