Received: 6 November 2023 | Accepted: 17 November 2023

DOI: 10.1111/ics.12935

International Journal of Cosmetic Science
SOCIÉTÉ FRANÇAISE DE COSMÉTOLOGIE

ORIGINAL ARTICLE

Investigation of piroctone olamine delivery to the skin from single, binary and ternary solvent systems

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Funding information
Biotechnology and Biological Sciences Research Council, Grant/Award Number: BB/T508962/1; Unilever

Abstract

Objective: Disruption of the protective stratum corneum barrier increases the skin's vulnerability to microorganisms and facilitates conditions such as dandruff. Dandruff is a disorder of the scalp that causes increased scaling of the SC and is associated with Malassezia fungus. Consequently, many anti-dandruff commercial products use anti-fungal active ingredients such as piroctone olamine also known as Octopirox (OPX). OPX is an active ingredient used in a number of topical preparations for the management of dandruff. The characterization of the physicochemical properties of OPX was previously reported. The aim of the present work was to investigate a range of solvent systems for their effects on OPX interaction with human skin.

Methods: The solvents used in this study were propylene glycol (PG), diethylene glycol monoethyl ether or Transcutol® (TC), PG monolaurate (PGML), isopropyl myristate (IPM), caprylic/capric triglyceride or Labrafac™ Lipophile WL 1349 (LAB), PG caprylate or Capryol® 90 (CAP), isostearyl isostearate (ISIS) and Plurol® Oleique CC 497 (PIOI). The single solvent systems evaluated were PG, TC, PGML, IPM, ISIS and CAP. For the binary solvent systems, PG and TC were examined. Ternary solvent systems consisted of: PG, TC and LAB; PG, PGML and LAB; and PG, TC and IPM. The concentration of OPX used was 1% (w/v). Heat-separated human epidermis was used for 24 h permeation experiments performed under finite dose conditions; mass balance studies were also conducted.

Results: For the six single solvents examined no permeation was evident. Skin permeation of OPX was observed for binary and ternary solvent systems. The highest permeation for all PG:TC binary solvent system ratios tested was from the PG:TC (75:25) system. For the ternary solvent systems investigated, highest cumulative permeation of OPX was observed for PG:PGML:LAB (60:30:10). Considering all systems, PG:TC (75:25) delivered the greatest amount of OPX through the skin. Although OPX is deposited in the skin following the application of neat solvents, higher skin retention values were generally observed for binary and ternary systems.

Conclusion: To our knowledge, this is the first study to examine the permeation behaviour of OPX for a range of single, binary and ternary solvent systems.

KEYWORDS
dandruff, finite dose, formulation, in vitro, piroctone olamine (OPX), skin delivery

Résumé

Objectif: La perturbation de la barrière protectrice de la couche cornée augmente la vulnérabilité de la peau aux micro-organismes et facilite des affections telles que les pellicules. Les pellicules sont un trouble du cuir chevelu qui provoque une augmentation de la desquamation de la couche cornée et qui est associé au champignon Malassezia. Par conséquent, de nombreux produits commerciaux antipelliculaires utilisent des principes actifs antifongiques, tels que la piroctone olamine, également appelée Octopirox (OPX). L'OPX est un principe actif utilisé dans un certain nombre de préparations topiques pour la prise en charge des pellicules. La caractérisation des propriétés physicochimiques de l'OPX a été précédemment rapportée. L'objectif de ce travail était d'étudier un éventail de systèmes de solvants pour leurs effets sur l'interaction de l'OPX avec la peau humaine.

Méthodes: Les solvants utilisés dans cette étude étaient le propylène glycol (PG), l'éther monoéthylique de diéthylèneglycol ou Transcutol® (TC), le monolaurate de propylène glycol (PGML), le myristate d'isopropyle (IPM), le triglycéride caprylique/caprique ou Labrafac™ lipophile WL 1349 (LAB), le caprylate de propylène glycol ou Capryol® 90 (CAP), l'isostéarate d'isostéaryle (ISIS) et Plurol® Oleique CC 497 (PIOI). Les systèmes à solvant unique évalués étaient le PG, le TC, le PGML, l'IPM, l'ISIS et le CAP. Pour les systèmes de solvants binaires, le PG et le TC ont été examinés. Les systèmes de solvants ternaires comprenaient : PG, TC et LAB ; PG, PGML et LAB ; et PG, TC et IPM. La concentration d'OPX utilisée était de 1 % (p/v). L'épiderme humain séparé par la chaleur a été utilisé pour des expériences de perméation de 24 heures réalisées dans des conditions de dose finie ; des études d'équilibre de masse ont également été menées.

Résultats: Pour les six solvants uniques examinés, aucune perméation n'était manifeste. Une perméation cutanée de l'OPX a été observée pour les systèmes de solvants binaires et ternaires. La perméation la plus élevée pour tous les rapports du système de solvant binaire PG:TC testés a été obtenue avec le système PG:TC (75:25). Pour les systèmes de solvants ternaires étudiés, la perméation cumulée la plus élevée d'OPX a été observée pour PG:PGML:LAB (60:30:10). Parmi tous les systèmes, PG:TC (75:25) a délivré la plus grande quantité d'OPX à travers la peau. Bien que l'OPX se dépose dans la peau après l'application de solvants purs, des valeurs de rétention cutanée plus élevées ont généralement été observées pour les systèmes binaire et ternaire.

Conclusion: À notre connaissance, il s'agit de la première étude visant à examiner le comportement de perméation de l'OPX pour un éventail de systèmes de solvants uniques, binaires et ternaires.

INTRODUCTION

The scalp is a unique skin area of the body consisting of five layers: the skin, connective tissue, galea aponeurotica, loose areolar connective tissue and the pericranium [1]. This part of the body is characterized by thick skin and a high follicular density with a large number of sebaceous glands [2]. The presence of these characteristics in combination with a dark and warm environment, makes the scalp more prone to fungal infections such as dandruff [3]. Dandruff is defined as a non-inflammatory, condition characterized by increased scaling of the scalp [4] that also causes itching. Almost 50% of the world's population is affected with dandruff at some point, and thus it is one of the most common dermatological skin conditions [5].

Dandruff and seborrheic dermatitis are thought to be caused by Malassezia yeast spp, sebaceous secretions and the individual's susceptibility [6]. Malassezia are a type of basidiomycetous fungi and are part of the normal skin flora of a variety of animals [7]. Most species are lipophilic, and owing to their lipophilicity they tend to colonize sites of the body that are seborrheic such as the scalp [8]. Certain skin diseases are primarily associated with Malassezia yeasts and 17 different species of Malassezia have so far been identified [9]. Among the identified species, *Malassezia restricta* is the most dominant on human skin [10–13]. Studies have proposed that several Malassezia species including *M. restricta* require lipids and that the breakdown of sebaceous triglycerides releases irritating free fatty acids that result in inflammation and scalp flaking [14, 15]. Some Malassezia species including *M. restricta* utilizes the carbon in lipid-rich areas for growth, for the colonization of Malassezia spp, sebum would be ideal [16].

Piroctone olamine or Octopirox® (OPX) is a cytostatic, anti-fungal agent widely used in cosmetic products such as anti-dandruff rinse-off shampoos (Figure 1) [17]. The physicochemical properties of OPX are described in Table 1 [18]. The exact mechanism of action of OPX is not fully understood [17]. One theory proposes that OPX enters the cell wall of the fungal cells and chelates with iron (III) ions [19]. The creation of these complexes leads to the inhibition of energy metabolism in the mitochondria resulting in fungicidal effects [20]. In addition, OPX was shown to exhibit inhibitory action on collagenolytic activity [21] with other authors noting that the compound may have the potential to protect against skin ageing [22].

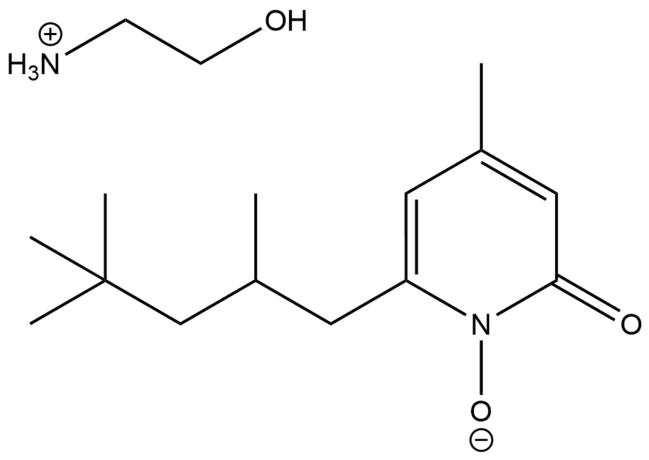

FIGURE 1 Structure of OPX. OPX, Octopirox.

TABLE 1 The physicochemical properties of Octopirox (OPX) [18].

Physicochemical properties of OPX	
Molecular weight ($g\,Mol^{-1}$)	298.42
Formula	$C_{16}H_{30}N_2O_3$
Log $D_{(O/PBS)}$ (pH 7.4 [buffered])	1.84 ± 0.08
Melting point (°C)	132.4
pK_a	
Piroctone moiety	6.87
Ethanolamine (olamine)	9.55

In a study involving 21 subjects with severe dandruff, Piérard-Franchimont et al. showed that a shampoo formulation containing 1% OPX induced significant reductions in the colonization of Malassezia yeast and scalp scaliness [23]. Lodén and Wessman investigated the use of shampoos containing 0.75% OPX/2% salicylic acid compared with 1% zinc pyrithione in a double-blind, randomized study with 19 subjects. The combination of OPX and salicylic acid was noted to be more effective in reducing the area and severity of scaling than zinc pyrithione [24].

To effectively target superficial fungal colonization such as *Malassezia* spp, the anti-fungal agent must be delivered to the SC, hair follicles or nails with limited permeation into the systemic circulation [25]. Although OPX is widely used in commercial formulations, there is little reported information about the skin uptake of OPX from such products and there are no studies that have examined OPX permeation in human skin. The aim of the present study was therefore to investigate the interaction of OPX with the skin in vitro following application in a range of solvents including single, binary and ternary systems. Solvents were selected based on their use in commercial skin and shampoo formulations and included propylene glycol (PG), diethylene glycol monoethyl ether as Transcutol® P (TC), PG caprylate as Capryol® 90 (CAP), PG monolaurate (PGML), caprylic/capric triglycerides as Labrafac™ lipophile WL 1349 (LAB), polyglyceryl-3 oleate as Plurol® Oleique CC 497 (PIOI), isostearyl isostearate (ISIS) and isopropyl myristate (IPM).

MATERIALS AND METHODS

Materials

OPX was donated by Unilever (Port Sunlight, UK). TC, CAP, PGML, LAB and PIOI were kind gifts from Gattefossé, (St. Priest, France). ISIS and IPM were provided as a free sample by Croda (Goole, UK). PG, HPLC grade deionized water (DI), acetonitrile (ACN), methanol (MeOH) and trifluoroacetic acid (TFA) were purchased from Fisher Scientific (Loughborough, UK). Phosphate Buffered Saline (PBS) Tablets (Dulbecco A) were purchased from Oxoid, Basingstoke, UK. Brij® O20 was purchased from Sigma–Aldrich (Dorset, UK).

Solubility studies and solubility parameter calculation

Solubility was determined as reported previously [16]. Briefly, OPX was added to saturation to 0.5 mL of solvent(s) in sample tubes and kept at 32 ± 1°C for 48 h in a JB Nova thermostatically controlled water bath (Grant, UK) equipped with an HP 15 stirring system (Variomag®, USA). Samples were centrifuged (5415 R centrifuge Eppendorf®, UK) at 13 200 rpm for 30 min at 32°C, and the supernatant was withdrawn and suitably diluted to lie within the range of the calibration curve. Samples were analysed by HPLC. All measurements were conducted in triplicate. The solubility parameter was calculated with Molecular Modelling Pro® (Version 6.3.3).

In vitro permeation experiments

Piroctone olamine has been approved for use in cosmetic products at a maximum concentration of 1% (w/v) in rinse-off products [26]. This concentration of active was used for all in vitro permeation studies in the present work. The excised human epidermis used in in vitro permeation experiments was prepared by the heat separation method as reported previously [27]. The experiments were performed as finite dose studies with an application volume of 10 µL. The receptor fluid used in the Franz cells was PBS with the addition of 6% (w/v) Brij® O20 to maintain sink conditions. The temperature was controlled with a JB Nova water bath (Grant, UK) and the receptor fluid in the Franz cells was stirred continuously with a HP 15 stirring system (Variomag®, USA). The Franz cells were placed in the water bath until the skin surface reached 32 ± 1°C. Solutions of 1% w/v OPX in the selected solvent system were subsequently applied to each cell. Permeation experiments were conducted for 24 h, followed by mass balance studies. The time points at which 200 µL samples were collected from the receptor compartment were 0, 0.5, 1, 2, 4, 8, 12, 24 h; all samples were analysed by high-performance liquid chromatography (HPLC).

Mass balance studies

After 24 h, each cell was washed five times with 1 mL of the appropriate solvent. Each cell was swabbed with a cotton bud and then cut to fit 2 mL Eppendorf microcentrifuge tubes (Eppendorf®, UK). After swabbing, the cells were disassembled, and the skin was cut into small pieces and inserted into separate microcentrifuge tubes. All sample tubes were shaken for 24 h at 32 ± 1°C. After 24 h, all extraction samples were subjected to two cycles of 15 min of ultrasonication (Ultrasonic Cleaner USC-THD VWR International, USA) followed by 15 min of centrifugation (5415 R centrifuge Eppendorf®, UK).

High-performance liquid chromatography analysis

HPLC analysis was performed using a 1100 series HPLC (Agilent, USA) with a reverse phase C_{18} column (Poroshell 120 EC-C18, 100 × 4.6 mm × 4 µm; Agilent, USA). The mobile phase was a gradient mixture of mobile phase A consisting of 95:5 v/v water:ACN with 0.1% TFA and mobile phase B consisting of 95:5 v/v ACN:water with 0.1% TFA as described in Table 2. The column temperature, mobile phase flow rate and injection volume were set at 32°C, 1 mL min^{-1} and 20 µL, respectively. The detection wavelength was set to 305 nm. Calibration curves in the range of 0.5–100 µg ml^{-1} were prepared in ACN:water (50:50 v/v) and the HPLC method was validated as per ICH guidelines [28].

Statistical analysis

Statistical analysis of the data collected was conducted using GraphPad Prism Statistics software (GraphPad

TABLE 2 Mobile phase gradient for high-performance liquid chromatography analysis of Octopirox.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	50	50
4.0	30	70
6.0	20	80
8.0	50	50
15.0	50	50

Software LLC, San Diego, CA, USA). The assessment of normality was conducted with the Shapiro–Wilk test. Data were evaluated using one-way analysis of variance (one-way ANOVA) and grouped data was evaluated using two-way analysis of variance (two-way ANOVA). A post hoc multiple comparisons test was used to compare the means between groups using the Tukey test.

RESULTS AND DISCUSSION

Solubility parameter of single, binary and ternary solvent systems

Figure 2 shows a plot of the calculated solubility parameter of the solvents investigated against the saturated solubility of OPX. The range of OPX solubility in the solvents used for this study ranged from 5.2 ± 0.07 to $624.96 \pm 25.49\,mg\,ml^{-1}$. The solvent system with the highest OPX solubility was found to be PG:PGML:LAB (60:30:10). In solvent systems that contain PG, the solubility of OPX was found to increase with higher amounts of PG. The solubility parameter values determined ranged from 8.1 to 14.07 $(cal/cm^3)^{1/2}$.

Single solvent systems

The skin permeation of OPX was investigated for six single solvents and mass balance results are also reported (Figure 3). OPX did not permeate the skin and was not detected in the receptor compartment. As OPX is a salt it is possible that limited or slow diffusion across the SC may account for the absence of permeation [29]. The three highest skin extraction values for OPX were obtained for PGML, TC and PG, with values of $10.09\% \pm 0.97\%$, $6.92\% \pm 1.47\%$ and $5.12\% \pm 0.55\%$ of the applied dose respectively. The results for PGML were significantly higher compared with the other solvent systems, ($p < 0.05$).

The amount of OPX recovered from the skin surface for all single solvent systems ranged from 81.69% to 91.54% of the dose applied (Figure 3). With the exception of PG, significantly higher amounts of OPX were recovered from the skin surface for CAP compared with the other solvents ($p < 0.05$). The highest amount of OPX recovered was for PG with a total recovery value of $93.95\% \pm 1.96\%$. The recovery percentage of OPX in PG was significantly higher compared with TC and ISIS with total recovery percentage values of $89.70\% \pm 2.56\%$ and $89.66\% \pm 2.81\%$ respectively ($p < 0.05$). When the recovery percentage of OPX in PG was compared with other solvent systems, the values were statistically similar ($p > 0.05$).

PGML is a fatty acid ester used as a solubilizer for active ingredients with poor solubility and it is also used as a cosurfactant. Previous reports have also demonstrated the potential of PGML to function as a skin penetration enhancer [30–32]. In the present study, the highest amount of OPX extracted from the skin was from PGML as shown in Figure 3. Parisi et al. investigated the topical delivery of hexamidine in a range of single and binary solvent systems. The researchers concluded that the addition of PGML clearly improved the topical delivery of both

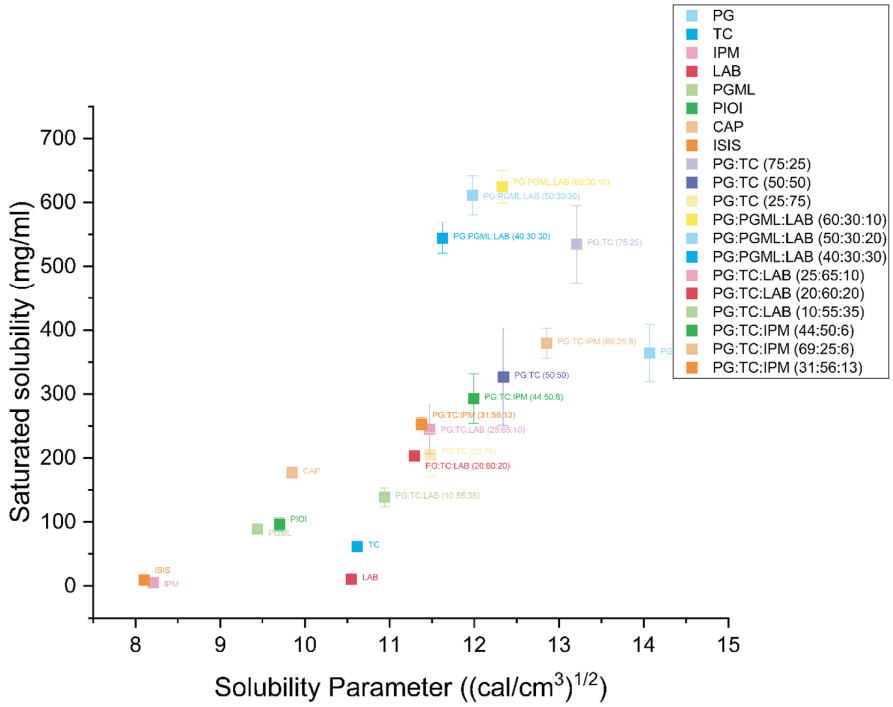

FIGURE 2 Plot of solvent solubility parameter against saturated solubility of OPX in the solvents and mixtures used at $32 \pm 1°C$ ($n = 3$, mean ± SD). OPX, Octopirox.

hexamidine diisethionate and its dihydrochloride form compared to the neat solvents alone [32]. The authors also suggested that the free fatty acid fraction of the ester may play a role in promoting skin uptake of these actives.

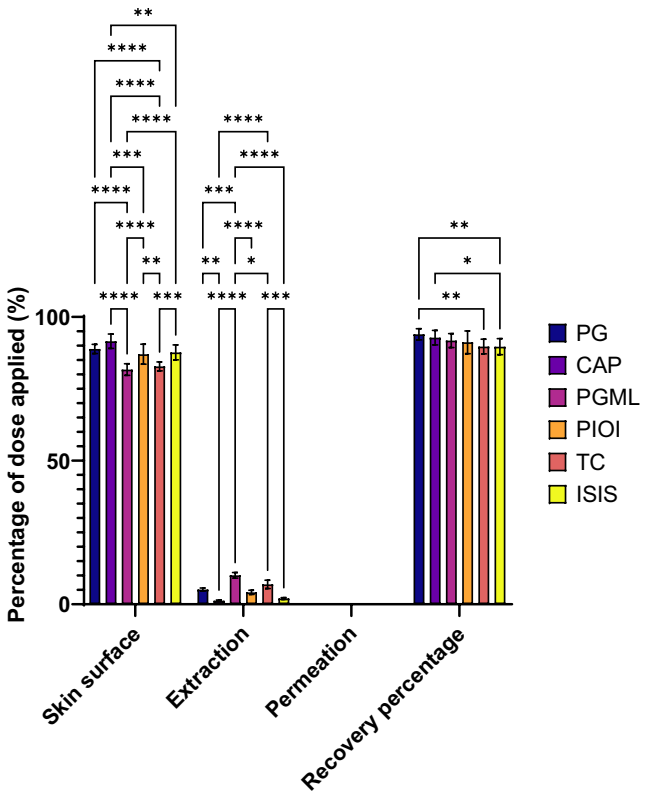

FIGURE 3 Mass balance studies for OPX in different single solvent systems using heat-separated human epidermis after 24 h permeation studies. $n = 6$, mean ± SD. Asterisks meaning: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$ and **** = $p \leq 0.0001$. OPX, Octopirox.

TC is a monoethyl ether of diethylene glycol that has been reported to increase drug solubility and promote skin penetration [33]. TC is thought to penetrate the stratum corneum and strongly interact with water along the intercellular path. This may then lead to a decrease in skin barrier function due to the modification of proteins and lipids and therefore more active permeation [34]. Iliopoulos et al. investigated the influence of neat solvents on the permeation of niacinamide. The researchers noted that TC promoted the greatest skin penetration of niacinamide but did not promote skin retention compared with other solvents studied [35]. As we have noted previously the results reported here confirm that solvents such as TC may not always act as skin penetration enhancers and in some cases might actually retard skin delivery.

Binary solvent systems

PG:TC

OPX permeation was further investigated using binary solvent systems of PG and TC. It is interesting to note that while there was no permeation of OPX from single solvent systems, OPX did permeate through the skin for all binary solvent systems (Figure 4). For PG:TC (25:75) and PG:TC (75:25), permeation of OPX was detected 1 h after application. For PG:TC (50:50), permeation was detected 2 h after application. At 24 h, a significantly higher amount of OPX was delivered from PG:TC (75:25) compared with all other PG:TC binary systems ($p < 0.05$) with cumulative permeation values of 10.50 ± 1.49 μg/cm^2. The comparatively high amounts of PG for the PG:TC (75:25) system

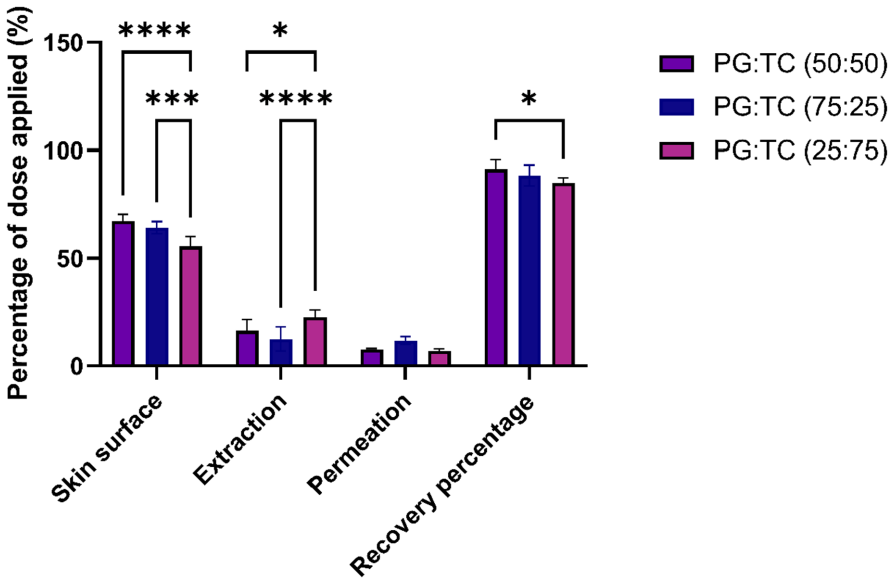

FIGURE 4 Mass balance studies for OPX in PG:TC vehicles in human skin after 24 h. $n = 6$, mean ± SD. Asterisks meaning: * = $p \leq 0.05$, *** = $p \leq 0.001$ and **** = $p \leq 0.0001$. OPX, Octopirox.

may be the driver for the higher OPX skin penetration. Notably the amount of OPX that permeated for PG:TC (50:50) was $7.09 \pm 0.43\,\mu g/cm^2$ compared with a value of $6.68 \pm 0.88\,\mu g/cm^2$ for PG:TC (25:75). However, these values are not significantly different ($p > 0.05$).

For PG:TC, the amount of OPX recovered from the surface of the skin was 55.50% ± 4.64% to 67.26% ± 3.21% of the dose applied. For PG:TC (50:50) and PG:TC (75:25), significantly higher recovery of OPX from the skin surface was noted compared with PG:TC (25:75) ($p < 0.05$). The recovery values of OPX for PG:TC (50:50) and PG:TC (75:25) from the skin surface and values for retention of OPX within the skin were comparable ($p > 0.05$). The amount of OPX extracted from the skin for the PG:TC (25:75) solvent system was 22.52% ± 3.44% of the applied dose. This was significantly higher when compared with PG:TC (50:50) and PG:TC (75:25) ($p < 0.05$), with extraction values of 16.28% ± 5.27% and 12.46% ± 5.59% of the dose applied respectively. There were no differences in the percentage permeation of OPX between all PG:TC systems ($p > 0.05$). The percentage permeation of OPX dose applied in PG:TC (75:25) was 11.75% ± 1.81% compared with 7.72% ± 0.36% and 6.96% ± 0.96% for PG:TC (50:50) and 25:75 respectively. Thus, these binary systems may be ranked as follows with reference to permeation: PG:TC (75:25) > PG:TC (50:50) > PG:TC (25:75).

The inclusion of additional solvents in the case of binary and ternary solvent systems has been suggested to produce a synergistic effect on the permeation of various compounds [30,32,36,37]. Zhang et al. investigated the permeation of niacinamide from various binary and ternary formulations in porcine and human skin and an artificial membrane model. Higher amounts of niacinamide permeated in human skin for binary solvent systems of PG with a fatty acid compared with neat solvents [36]. Kung et al. explored single, binary and ternary solvent systems for the delivery of methadone. The researchers reported that PG:TC (50:50) delivered significantly more methadone through human skin after 24 h compared with d-limonene and combinations of d-limonene with ethyl oleate, TC and octyl salicylate ($p < 0.05$) [37]. It was suggested that the promotion of permeation from the use of the binary solvent system PG:TC might bone attributed to PG increasing the permeation of both the active compound and TC. The higher uptake of TC may also lead to additional permeation of the active compound [37].

Ternary solvent systems

PG:PGML:Lab

Building on the results reported in the previous section, the solvents PG, PGML and LAB were selected as a ternary solvent system. The specific ratios were identified based on miscibility evaluation (data not shown). Permeation of OPX was observed for all ternary solvent systems. Permeation was not observed until the 8 h timepoint (data not shown). At the 24 h timepoint, significantly more OPX permeated for PG:PGML:LAB (60:30:10) compared with the other PG:PGML:LAB vehicles examined ($p < 0.05$). Higher OPX permeation was also evident for PG:PGML:LAB (50:30:20) compared with PG:PGML:LAB (40:30:30), $p < 0.05$. Values for OPX skin penetration were $7.05 \pm 0.57\,\mu g/cm^2$ for PG:PGML:LAB (60:30:10), compared with 4.29 ± 0.28 and $3.69 \pm 0.20\,\mu g/cm^2$ for PG:PGML:LAB (50:30:20) and for PG:PGML:LAB (40:30:30) respectively.

The mass balance results for the PG:PGML:LAB vehicles are shown in Figure 5. Overall, recoveries for OPX for all PG:PGML:LAB vehicles were >88% of the dose applied. The differences in recovery percentages between

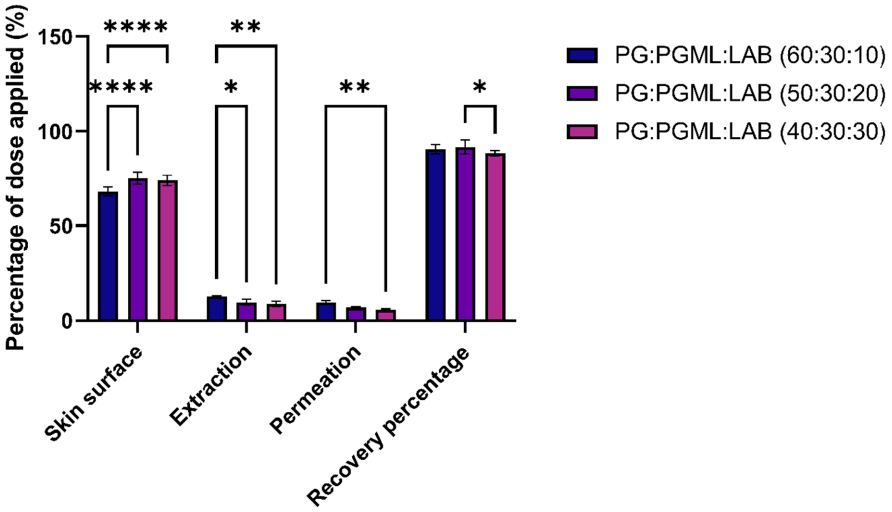

FIGURE 5 Mass balance studies for OPX in PG:PGML:LAB vehicles in human skin after 24 h permeation studies. $n = 6$, mean ± SD. Asterisks meaning: * = $p \leq 0.05$, ** = $p \leq 0.01$ and **** = $p \leq 0.0001$. OPX, Octopirox; PG, propylene glycol; PGML, propylene glycol monolaurate.

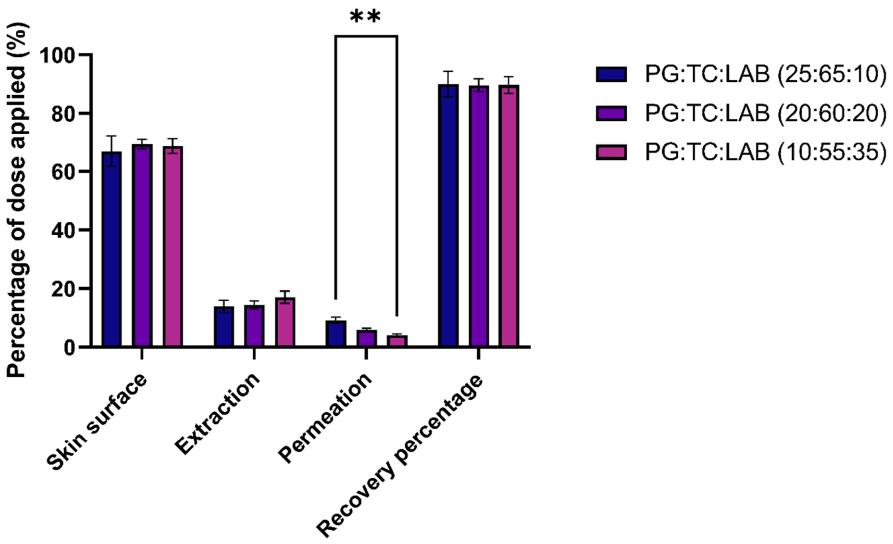

FIGURE 6 Mass balance studies for OPX for PG:TC:LAB vehicles in human skin after 24 h permeation studies. $n = 6$, mean ± SD. Asterisks meaning: ** = $p \leq 0.01$. OPX, Octopirox; PG, propylene glycol; TC, Transcutol®.

PG:PGML:LAB (60:30:10) and the other ratios were not significant ($p > 0.05$). However, the recovery of OPX for PG:PGML:LAB (50:30:20) was significantly higher compared with PG:PGML:LAB (40:30:30) ($p < 0.05$). In terms of skin surface recovery, both the PG:PGML:LAB (50:30:20) and PG:PGML:LAB (40:30:30) deposited significantly more OPX on the skin surface compared with PG:PGML:LAB (60:30:10) ($p < 0.05$). For PG:PGML:LAB (60:30:10), the extraction values were also significantly higher compared with the other vehicles ($p < 0.05$), with a recovery of 12.80% ± 0.48% of the dose applied. The corresponding values for PG:PGML:LAB (50:30:20) and PG:PGML:LAB (40:30:30) were 9.74% ± 1.58% and 8.85% ± 1.37%. With regards to permeation, PG:PGML:LAB (60:30:10) promoted significantly more OPX skin penetration compared with PG:PGML:LAB (40:30:30) ($p < 0.05$). 9.63% ± 0.89% of the dose of OPX permeated for PG:PGML:LAB (60:30:10), compared with 5.80% ± 0.36% of the dose applied for PG:PGML:LAB (40:30:30). The percentage permeation of OPX from PG:PGML:LAB (60:30:10) was not significantly higher than corresponding values for PG:PGML:LAB (50:30:20) ($p > 0.05$).

The most frequently utilized glycol in topical and transdermal products is PG. Although the mechanism of action of PG is not fully understood, it is commonly used as a co-solvent and is reported to be a permeation enhancer [33]. The mass balance study in this work indicates that increasing amounts of PG results in increased permeation and skin retention of OPX. Similar findings were reported by Kung et al. for methadone and Trottet et al. for loperamide [37, 38]. Iliopoulos et al. conducted in vitro permeation experiments with 3-O-ethyl l-ascorbic acid (EA) in a range of binary and ternary solvent systems. High EA delivery corresponded to high amounts of PG in both binary and ternary solvent systems [30].

PG:TC:Lab

At 24 h, the cumulative permeated amounts of OPX from PG:TC:LAB vehicles ranged from 3.38 to 7.42 μg/cm^2. The highest cumulative amount of OPX that permeated was for PG:TC:LAB (25:65:10) with a value of 7.42 ± 0.99 μg/cm^2. This value is significantly higher than values observed for the other PG:TC:LAB systems ($p < 0.05$). PG:TC:LAB (20:60:20) also promoted significantly greater permeation of OPX than PG:TC:LAB (10:55:35) ($p < 0.05$); the corresponding permeation values were 4.91 ± 0.44 μg/cm^2 and 3.38 ± 0.40 μg/cm^2 respectively.

The results for the mass balance study of OPX for PG:TC:LAB vehicles are summarized in Figure 6. For PG:TC:LAB (20:60:20) the percentage of the dose applied recovered from the skin surface was 69.32% ± 1.61%. However, there are no significant differences for the three systems with reference to deposition of OPX on the skin ($p > 0.05$). The highest OPX extraction from within the skin was observed for PG:TC:LAB (10:55:35) with a value of 17.03% ± 2.10% of the dose applied. Similar to the results from the skin surface, the results were not significantly different for the three vehicles ($p > 0.05$). The highest permeation of OPX was from PG:TC:LAB (25:65:10) with a value of 9.19% ± 1.07% of the dose applied. OPX permeated significantly more for this system compared with PG:TC:LAB (10:55:35) ($p < 0.05$) but permeation was not significantly higher ($p > 0.05$) when compared with PG:TC:LAB (20:60:20).

PG:TC:IPM

As for the other ternary systems, for the PG:TC:IPM vehicles the ratios selected were based on miscibility evaluation. At 24 h, the highest permeated amount of OPX

was observed for PG:TC:IPM (44:50:6) with a value of $4.00 \pm 0.46\,\mu g/cm^2$ compared with $3.66 \pm 0.45\,\mu g/cm^2$ and $3.65 \pm 0.30\,\mu g/cm^2$ for PG:TC:IPM (69:25:6) and PG:TC:IPM (31:56:13) respectively. However, permeation values were not statistically significantly different ($p > 0.05$).

Figure 7 shows the results of the mass balance studies for the PG:TC:IPM systems. The highest amount of OPX was deposited on the skin surface for PG:TC:IPM (44:50:6) with a value of $68.13\% \pm 3.63\%$ of the dose applied. The corresponding values for PG:TC:IPM (69:25:6) and PG:TC:IPM (31:56:13) were $65.93\% \pm 3.86\%$ and $64.32\% \pm 6.60\%$ respectively. However, these values were not statistically significantly different ($p > 0.05$). In terms of extraction from within the skin, both PG:TC:IPM (44:50:6) and PG:TC:IPM (31:56:13) promoted significantly higher OPX retention compared with PG:TC:IPM (69:25:6) ($p < 0.05$). The percentage of the dose applied

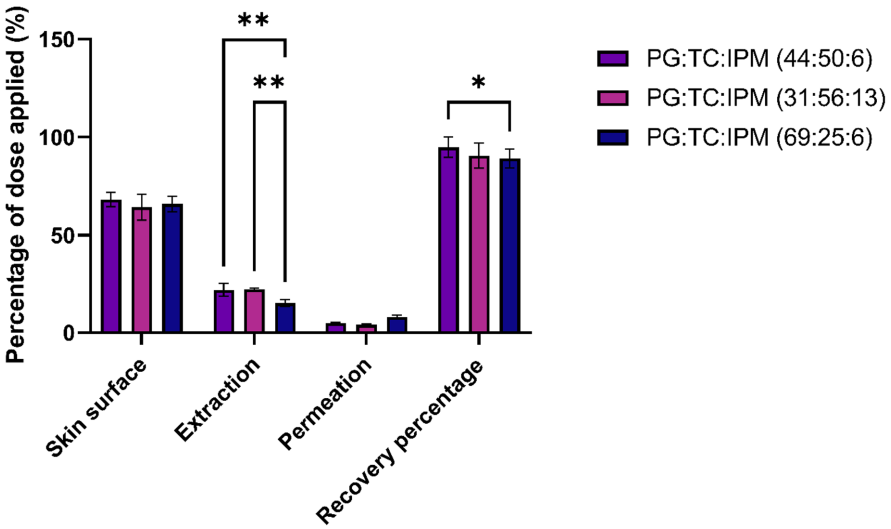

FIGURE 7 Mass balance studies for OPX in different ratios of PG:TC:IPM as a ternary solvent system using heat-separated human epidermis after 24 h permeation studies. $n = 6$, mean ± SD. Asterisks meaning: * = $p \leq 0.05$, ** = $p \leq 0.01$. IPM, isopropyl myristate; OPX, Octopirox; PG, propylene glycol; TC, Transcutol®.

TABLE 3 Mass balance results of OPX in single, binary and ternary solvent systems after 24 h permeation studies ($n = 6$, mean ± SD).

	Percentage of applied dose of OPX (%)		
Solvent system	**Recovered from skin surface**	**Extracted from inside the skin**	**Permeation**
PG	88.84 ± 1.60	5.12 ± 0.55	ND
CAP	91.55 ± 2.48	1.25 ± 0.23	ND
PGML	81.69 ± 2.02	10.09 ± 0.97	ND
PIOI	87.02 ± 3.47	4.15 ± 0.72	ND
TC	82.78 ± 1.57	6.92 ± 1.50	ND
ISIS	87.66 ± 2.60	2.0 ± 0.33	ND
PG:TC (75:25)	64.23 ± 2.81	12.46 ± 5.59	11.75 ± 1.81
PG:TC (50:50)	67.26 ± 3.21	16.28 ± 5.27	7.72 ± 0.36
PG:TC (25:75)	55.50 ± 4.64	22.52 ± 3.44	6.9 ± 0.96
PG:PGML:LAB (60:30:10)	68.11 ± 2.44	12.80 ± 0.43	9.63 ± 0.89
PG:PGML:LAB (50:30:20)	75.10 ± 3.34	9.74 ± 1.44	6.92 ± 0.54
PG:PGML:LAB (40:30:30)	73.89 ± 2.77	8.85 ± 1.49	5.80 ± 0.36
PG:TC:LAB (25:65:10)	66.92 ± 5.22	13.93 ± 2.15	9.19 ± 1.07
PG:TC:LAB (20:60:20)	69.32 ± 1.61	14.44 ± 1.35	5.91 ± 0.55
PG:TC:LAB (10:55:35)	68.60 ± 2.51	17.03 ± 2.10	4.10 ± 0.41
PG:TC:IPM (69:25:6)	65.93 ± 3.86	15.11 ± 2.05	8.10 ± 0.88
PG:TC:IPM (44:50:6)	68.13 ± 3.63	21.87 ± 3.32	4.83 ± 0.52
PG:TC:IPM (31:56:13)	64.32 ± 6.60	22.18 ± 0.67	4.11 ± 0.30

Abbreviation: ND, not detected.

extracted from the skin was 15.11% ± 2.05% for PG:TC:IPM (69:25:6) compared with 21.87% ± 3.32% for PG:TC:IPM (44:50:6) and 22.18% ± 0.67% for PG:TC:IPM (31:56:13).

Table 3 shows the results from the mass balance studies from all the solvent systems tested. The amount of OPX that remained on the skin surface ranged from 55.50% ± 4.64% to 91.55% ± 2.48% of the dose applied. For OPX skin extraction, values ranged from 1.25% ± 0.23% to 22.52% ± 3.44% of the dose applied. As noted, no permeation was observed for single solvents. For binary and ternary solvent systems, the permeated values of OPX range from 4.10% ± 0.41% to 11.75% ± 1.81% dose applied. As well as enhancing skin permeation it is evident that most of the binary and ternary systems also promote OPX skin retention.

CONCLUSION

Only one report of the skin permeation of OPX in vivo in rodents has been published to date [39]. To our knowledge, this is the first comprehensive evaluation of OPX penetration and distribution in human skin in vitro. In the present work, while no permeation was observed for simple vehicles, more complex binary and ternary systems generally promoted both skin penetration as well as skin retention. This may reflect changes in the solubility and partition behaviour of OPX in the SC or structural changes within the SC itself with increasing vehicle complexity. As differing amounts of retention and penetration were observed in different systems the potential for vehicle manipulation to preferentially promote skin retention rather than skin penetration should be explored further. This will require monitoring of the vehicle as well as the active. Appropriate techniques would be HPLC to monitor OPX and gas chromatography for the vehicle.

ACKNOWLEDGEMENTS

This work was supported by Unilever and the Biotechnology and Biological Sciences Research Council (BB/T508962/1).

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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REFERENCES

1. Tajran J, Gosman AA. Anatomy, head and neck, scalp. Treasure Island (FL): StatPearls Publishing; 2022.
2. Bin Saif GA, Ericson ME, Yosipovitch G. The itchy scalp – scratching for an explanation. Exp Dermatol. 2011;20(12):959–68.
3. Narshana M, Ravikumar P, Nanavati DB. An overview of dandruff and novel formulations as a treatment strategy. Int J Pharm Sci Res. 2018;9(2):417–31.
4. Manuel F. Is dandruff a disease? Int J Trichology. 2010;2(1):68.
5. Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: a comprehensive review. J Clin Investig Dermatol. 2015;3(2):1–22.
6. Park M, Cho YJ, Lee YW, Jung WH. Understanding the mechanism of action of the anti-dandruff agent zinc Pyrithione against *Malassezia restricta*. Sci Rep. 2018;8(1):12086.
7. Cabañes FJ. Malassezia yeasts: how many species infect humans and animals? PLoS Pathog. 2014;10(2):e1003892-e.
8. Thayikkannu AB, Kindo AJ, Veeraraghavan M. Malassezia-can it be ignored? Indian J Dermatol. 2015;60(4):332–9.
9. Velegraki A, Cafarchia C, Gaitanis G, Iatta R, Boekhout T. Malassezia infections in humans and animals: pathophysiology, detection, and treatment. PLoS Pathog. 2015;11(1):e1004523-e.
10. Xu Z, Wang Z, Yuan C, Liu X, Yang F, Wang T, et al. Dandruff is associated with the conjoined interactions between host and microorganisms. Sci Rep. 2016;6(1):24877.
11. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, et al. Topographic diversity of fungal and bacterial communities in human skin. Nature. 2013;498(7454):367–70.
12. Clavaud C, Jourdain R, Bar-Hen A, Tichit M, Bouchier C, Pouradier F, et al. Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. PLoS One. 2013;8(3):e58203.
13. Grimshaw SG, Smith AM, Arnold DS, Xu E, Hoptroff M, Murphy B. The diversity and abundance of fungi and bacteria on the healthy and dandruff affected human scalp. PLoS One. 2019;14(12):e0225796.
14. DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL. Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. J Investig Dermatol Symp Proc. 2005;10(3):295–7.
15. Ro BI, Dawson TL. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. J Investig Dermatol Symp Proc. 2005;10(3):194–7.
16. Bonardi A, Gratteri P, Nocentini A. Chapter 19 - Carbonic anhydrases from pathogens: fungal carbonic anhydrases and their inhibitors as potential antifungal agents. Carbonic anhydrases. Cambridge, MA, United States: Academic Press; 2019. p. 419–48.
17. do Couto FM, do Nascimento SC, Junior SF, da Silva VK, Leal AF, Neves RP. Antifungal activity of the piroctone olamine in experimental intra-abdominal candidiasis. Springerplus. 2016;5:468.
18. Tang CF, Paz-Alvarez M, Pudney PDA, Lane ME. Characterization of piroctone olamine for topical delivery to the skin. Int J Cosmet Sci. 2023;45(3):345–53.
19. Kim Y, Alpmann P, Blaum-Feder S, Kramer S, Endo T, Lu D, et al. Increased in vivo efficacy of lenalidomide by addition of piroctone olamine. In Vivo. 2011;25(1):99–103.
20. Youn HJ, Kim SY, Park M, Jung WH, Lee YW, Choe YB, et al. Efficacy and safety of cream containing climbazole/piroctone olamine for facial seborrheic dermatitis: a single-center, open-label split-face clinical study. Ann Dermatol. 2016;28(6):733–9.

21. Fukuda Y, Nakashima S, Ujiie T. The in vitro effect of a collagenolytic enzyme inhibitor on lesion development in root dentin. Am J Dent. 2009;22(2):115–21.
22. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012;4(3):308–19.
23. Piérard-Franchimont C, Uhoda E, Loussouarn G, Saint-Léger D, Piérard GE. Effect of residence time on the efficacy of antidandruff shampoos. Int J Cosmet Sci. 2003;25(6):267–71.
24. Lodén M, Wessman C. The antidandruff efficacy of a shampoo containing piroctone olamine and salicylic acid in comparison to that of a zinc pyrithione shampoo. Int J Cosmet Sci. 2000;22(4):285–9.
25. Lengert EV, Talnikova EE, Tuchin VV, Svenskaya YI. Prospective nanotechnology-based strategies for enhanced intra- and transdermal delivery of antifungal drugs. Skin Pharmacol Physiol. 2020;33(5):261–9.
26. SCCNPF. Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning piroctone olamine and its monoethanolamine salt. Brussels: European Commission; 2002.
27. Oliveira G, Hadgraft J, Lane ME. The influence of volatile solvents on transport across model membranes and human skin. Int J Pharm. 2012;435(1):38–49.
28. ICH. Validation of analytical procedures: text and methodology Q2(R1). 2005.
29. Zhang K, Chen M, Scriba GK, Abraham MH, Fahr A, Liu X. Human skin permeation of neutral species and ionic species: extended linear free-energy relationship analyses. J Pharm Sci. 2012;101(6):2034–44.
30. Iliopoulos F, Sil BC, Moore DJ, Lucas RA, Lane ME. 3-O-ethyl-l-ascorbic acid: characterisation and investigation of single solvent systems for delivery to the skin. Int J Pharm X. 2019;1:100025.
31. Haque T, Rahman KM, Thurston DE, Hadgraft J, Lane ME. Topical delivery of anthramycin I. Influence of neat solvents. Eur J Pharm Sci. 2017;104:188–95.
32. Parisi N, Paz-Alvarez M, Matts PJ, Lever R, Hadgraft J, Lane ME. Topical delivery of hexamidine. Int J Pharm. 2016;506(1–2):332–9.
33. Lane ME. Skin penetration enhancers. Int J Pharm. 2013;447(1–2):12–21.
34. Osborne DW, Musakhanian J. Skin penetration and permeation properties of Transcutol(R)-neat or diluted mixtures. AAPS PharmSciTech. 2018;19(8):3512–33.
35. Iliopoulos F, Sil BC, Monjur Al Hossain ASM, Moore DJ, Lucas RA, Lane ME. Topical delivery of niacinamide: influence of neat solvents. Int J Pharm. 2020;579:119137.
36. Zhang Y, Kung CP, Sil BC, Lane ME, Hadgraft J, Heinrich M, et al. Topical delivery of niacinamide: influence of binary and ternary solvent systems. Pharmaceutics. 2019;11(12):1–13.
37. Kung CP, Zhang Y, Sil BC, Hadgraft J, Lane ME, Patel B, et al. Investigation of binary and ternary solvent systems for dermal delivery of methadone. Int J Pharm. 2020;586:119538.
38. Trottet L, Merly C, Mirza M, Hadgraft J, Davis AF. Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride. Int J Pharm. 2004;274(1–2):213–9.
39. Black JG, Kamat VB. Percutaneous absorption of Octopirox. Food and Chemical Toxicology. 1988;26(1):53–8. https://doi.org/10.1016/0278-6915(88)90041-5

How to cite this article: Tang CF, Pudney PDA, Lane ME. Investigation of piroctone olamine delivery to the skin from single, binary and ternary solvent systems. Int J Cosmet Sci. 2023;00:1–11. https://doi.org/10.1111/ics.12935