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Topical delivery of climbazole to mammalian skin

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ABSTRACT

Dandruff is a common condition, affecting up to half the global population of immunocompetent adults at some time during their lives and it has been highly correlated with the over-expression of the fungus *Malassezia spp*. Climbazole (CBZ) is used as an antifungal and preservative agent in many marketed formulations for the treatment of dandruff. While the efficacy of CBZ *in vitro* and *in vivo* has previously been reported, limited information has been published about the uptake and deposition of CBZ in the skin. Hence, our aim was to investigate the skin permeation of CBZ as well as the influence of various solvents on CBZ skin delivery. Four solvents were selected for the permeability studies of CBZ, namely propylene glycol (PG), octyl salicylate (OSal), Transcutol® P (TC) and polyethylene glycol 200 (PEG). The criteria for selection were based on their wide use as excipients in commercial formulations, their potential to act as skin penetration enhancers and their favourable safety profiles. 1% (w/v) solutions of CBZ were applied under infinite and finite dose conditions using Franz type diffusion cells to human and porcine skin. In line with the topical use of CBZ as an antidandruff agent, comparatively low amounts of CBZ penetrated across the skin barrier (< 1% of the applied dose of CBZ). Finite dose studies resulted in a higher extraction of CBZ from human skin compared with infinite dose studies ($p < 0.05$). CBZ was also taken up to a higher extent in porcine skin (> 7-fold) compared with human skin ($p < 0.05$). Nevertheless, no statistical differences were observed in the amounts that permeated across the different membranes. These preliminary results confirm the potential of simple formulations of CBZ to target the outer layers of the epidermis. The PG and OSal formulations appear to be promising vehicles for CBZ in terms of overall skin extraction and penetration. Future work will expand the range of vehicles studied and explore the reasons underlying the retention of CBZ in the outer layers of the skin.

1. Introduction

Human skin is a formidable barrier protecting against the entry of foreign substances and preventing excessive water loss from the body (Hadgraft and Lane, 2005; Notman and Anwar, 2013; Proksch et al., 2008). Notwithstanding this, various formulation approaches have been developed to overcome the skin's inherent resistance to penetration of actives. Topical and transdermal delivery of drugs is also attractive compared with oral or intravenous administration, as first pass metabolism is avoided and patient compliance when using such formulations is generally higher (Zorec et al., 2013). Many diseases require the active to be targeted to the skin itself. This is the case for many antifungals used to treat skin infections, where the oral route would require comparatively high doses of drugs and may result in undesirable systemic effects (Glujoy et al., 2014).

Dandruff is a common scalp disorder causing excessive scaling of the scalp and impaired skin barrier function (Hay, 2011). The commensal fungus *Malassezia spp* is commonly found in the scalp, but excessive proliferation of this organism is highly correlated with the presence of dandruff (DeAngelis et al., 2005). Dandruff is generally treated using medicated shampoos incorporating one or more actives to target different stages of the condition. These shampoos can contain keratolytic agents (salicylic acid, sulfur), regulators of keratinisation (zinc, tar, steroids), antimicrobial agents (selenium sulphide, imidazole antifungals, hydroxypyridones) and naturopathic remedies (tea tree oil) (Piérard et al., 2000; Sanfilippo, 2006).

Climbazole (CBZ) is an imidazole antifungal agent used to manage dandruff in formulations such as shampoos or conditioners and as a preservative (Fig. 1). It has a molecular weight of 292.8 g mol^{-1} and a reported melting point of 95–97 °C and it acts by inhibiting the synthesis of ergosterol, a major component in fungal plasma membranes (SCCP, 2009). In addition, CBZ has been shown to upregulate keratinocyte differentiation promoting the expression of small-proline-rich proteins in primary keratinocytes (Pople et al., 2014). Bhogal et al.

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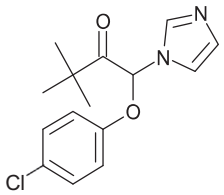

Fig. 1. Chemical structure of climbazole.

(2014) also reported inhibitory effects of CBZ on hair proteases involved in anchorage of the hair shaft in an *in vitro* study.

Ideally, formulations of CBZ should target the molecule to the *stratum corneum* and/or the hair follicles, for local effects on scalp microbiota. The minimum inhibitory concentration (MIC) for CBZ for various species of *Malassezia* associated with dandruff ranges from 125 to 62.5 μg ml^{-1} (*M. globosa* = *M. restricta* > *M. furfur*) as reported by Gokulshankar et al. (2011). Lower MICs have been reported by Youn et al. (2016), in the order of 1–2 μg ml^{-1}, showing a higher antifungal efficacy of CBZ compared with piroctone olamine, another antifungal agent used for the treatment of dandruff. Various authors have reported the *in vitro* activity and associated clinical outcomes of CBZ in the literature (Chen et al., 2015; Schmidt-Rose et al., 2011; Youn et al., 2016). Garrett et al. (2017) also investigated the deposition of CBZ and zinc pyrithione in porcine skin with stimulated Raman scattering microscopy. Higher penetration of CBZ into the hair shaft was observed compared with zinc pyrithione, the latter compound being deposited as crystals on the skin surface because of its lower lipid solubility. However, there is still limited information detailing skin delivery or efficiency of CBZ following topical application. As the molecule has a comparatively low melting point, low molecular weight and is lipophilic with a predicted log $P_{o/w}$ of 3.76 (SCCP, 2009), these properties suggest it is a suitable permeant for delivery to the skin (Hadgraft, 2004). The aim of the present work was to investigate the topical delivery of CBZ from four solvents. These vehicles were selected based on their solubility parameters, their application as skin penetration enhancers in other formulations, and their favourable safety profiles (Haque et al., 2017; Santos et al., 2010). Permeation and mass balance studies were conducted under infinite and finite dose conditions. Porcine ear skin has previously been proposed as an appropriate surrogate model for human scalp skin (Rolland et al., 2011). A secondary objective of the present work was, therefore, to investigate the differences in CBZ uptake and delivery in porcine and human tissues.

2. Materials and methods

2.1. Materials

Climbazole (Crinipan® AD, Symrise, Germany) was supplied by Unilever (Port Sunlight, UK). Propylene glycol (PG), polyethylene glycol 200 (PEG), 2-ethylhexyl salicylate (OSal) and 1,2-pentanediol, were purchased from Sigma Aldrich, UK. Transcutol® P (TC) was a gift from Gattefossé, France. HPLC grade water, acetonitrile (ACN) and methanol, 1,2-isopropanol, absolute ethanol (EtOH), sodium di-hydrogen orthophosphate, di-sodium hydrogen orthophosphate and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific, UK. For the permeation studies, phosphate buffered saline (PBS) tablets (pH 7.3 ± 0.2 at 25 °C) were supplied by Oxoid Limited, UK. High-vacuum grease was purchased from Dow Corning, Belgium and sodium azide was obtained from Fluka, Sigma Aldrich, UK. Full thickness porcine ear skin was obtained from a local abattoir as the waste product from pig carcasses that had been slaughtered the same day for food chain purposes. The full thickness skin was separated from the cartilaginous tissue at room temperature and then stored at −20 °C for short time until needed. Abdominal human skin was obtained following plastic surgery from a single female donor with institutional ethical approval and informed consent, provided by Ethical Tissue (Research Ethics Committee reference 07/H1306/98). The human skin was stored at −20 °C before use and the epidermis was heat separated before the studies as reported previously (Oliveira et al., 2012).

2.2. Melting point calculation

A Tzero™ press (TA instruments, USA) was used to seal the pan containing CBZ hermetically with its corresponding lid (TA instruments, USA) and an empty pan sealed with its lid was used as the reference. The samples were then heated from −20 to 140 °C at 10 °C min^{-1} under N_2 gas (50 ml min^{-1}) in a differential scanning calorimeter Q2000, (TA Instruments, USA). Data analysis was performed with TA Universal Analysis software.

2.3. Log $P_{o/w}$, Log $D_{o/w}$ and solubility determination

The Log $P_{o/w}$ was calculated with Molecular Modelling Pro® (Version 6.3.3) software (ChemSW, CA, USA). The Log $D_{o/w}$ for CBZ was determined experimentally using the shake flask method (OECD, 1995). Experiments were conducted at pH 5.0, 7.3 and 9.0 using a citric acid/trisodium citrate buffer, PBS tablets and sodium carbonate/sodium bicarbonate for the respective buffer solutions. N-octanol was used as the organic phase. All experiments were performed at room temperature (23 ± 2 °C). The amount of active in each phase in the different ratios was determined using HPLC to calculate the log $D_{o/w}$ as shown in Equation (1), where $C_{n\text{–octanol}}$ is the concentration in the organic phase and $C_{aqueous}$ the concentration in the relevant aqueous phase.

$$\log D_{ow} = \log\left(\frac{C_{n\text{ - octanol}}}{C_{aqueous}}\right) \quad (1)$$

An excess amount of added to 0.5 ml of solvent with stirring bars in flat bottom tubes. Tubes were capped, covered with Parafilm® (Bemis Company Inc., USA) and placed in a JB Nova thermostatically controlled water bath (Grant, UK) equipped with a HP 15 stirring system (Variomag®, USA) at 32° ± 1 °C for 48 h. The tubes were centrifuged at 13,200 rpm for 30 min at 32 °C, the supernatant was then carefully withdrawn and suitably diluted where necessary for analysis by HPLC. All measurements were conducted in triplicate.

2.4. Dynamic vapour sorption studies

A dynamic vapour sorption (DVS) apparatus (Surface Measurement Systems, UK) was used to study the mass differences resulting from evaporation or hydration of the candidate formulations. 15 mm quartz glass pans (reference and sample) connected to a microbalance accurate to 0.0001 mg were used to evaluate the mass variations. Temperature and relative humidity (RH) were controlled throughout the experiment at 32 ± 1 °C and 50 ± 2% RH, respectively. The DVS instrument was calibrated with a 100 mg weight and 10 μl of formulation was placed on the sample chamber for 24 h, i.e. solutions of 1% CBZ (w/v) in PG, OSal, TC and PEG 200. Data were recorded in 10 s intervals using DVSWin V3.01 (Surface Measurement System, UK).

2.5. Permeation studies

According to the current guidelines for topical use of CBZ in humans (SCCP, 2009), a concentration of up to 2% (w/v) in rinse-off hair products is allowed, and thus this concentration of active was used as a guide for *in vitro* studies. Initially, infinite dose studies were performed in human and porcine ear skin, followed by finite dose studies. In all cases, 1% (w/v) CBZ solutions in propylene glycol (PG), octyl salicylate (OSal), polyethylene glycol 200 (PEG) and Transcutol® P (TC) were applied to the membranes. 250 μl per cm^2 of skin surface were applied

for infinite dose studies while ~10 µl cm^{-2} of the formulation were applied for finite doses using a positive displacement pipette (Eppendorf®, UK), as described elsewhere (OECD, 2004b). Sink conditions were maintained throughout the experiments (Pellett et al., 1997). The permeation studies were performed using Franz type diffusion cells with a diffusion area of ~1 cm^2, accurately measured, as reported previously (Santos et al., 2010). Experiments were conducted for 48 h for the infinite dose studies and 24 h for the finite dose studies in a JB Nova thermostatically controlled water bath (Grant, UK) equipped with a HP 15 stirring system (Variomag®, USA). The upper surface temperature of the membrane was kept at 32 ± 1 °C. Samples were collected at 0, 8, 24 and 48 h for the infinite dose studies and 0, 8, 20 and 24 h for finite dose studies. 200 µl of receptor phase were withdrawn for each sample and the same volume of fresh PBS was replaced immediately after sample collection.

2.6. Mass balance studies

Both skin washing and extraction procedures were validated to confirm the recovery of the active was within the accepted ranges (OECD, 2004b). After the 24 or 48 h permeation studies (finite and infinite dose studies, respectively), the remaining volume in the donor (infinite dose studies) was removed and diluted accordingly to allow HPLC analysis. The surface of the skin was then washed with 1 ml of absolute ethanol (EtOH) four times for each cell in order to clean the surface of the skin thoroughly whilst avoiding extraction. The diffusion cells were disassembled, and the skin was placed in 2 ml microtest tubes (Eppendorf®, UK) for extraction with 1 ml of DMSO in a temperature-controlled oven at 32 ± 1 °C (Jouan Series Thermo Fisher Scientific, USA). After 24 h rotation in the oven, two 15 min cycles of sonication (Ultrasonic Cleaner USC-THD VWR International, USA) and centrifugation at 13,200 rpm (5415 R centrifuge Eppendorf®, UK) were performed. Following suitable dilution, the remaining volume in the donor, washings and the extraction supernatants were analysed by HPLC.

2.7. HPLC analysis

HPLC analysis was performed using an Agilent HPLC (Agilent 1100 series, USA) and a reverse phase C_{18} column (Kinetex 5 µm, pore size 100 Å, 50 x 4.6 mm; Phenomenex, UK). The mobile phase was phosphate buffer pH 7.4 ± 0.2 : Acetonitrile (40:60). The column temperature, mobile phase flow rate and injection volume were set at 30 °C, 1 ml min^{-1} and 10 µl, respectively. The detection wavelength was set to 220 nm. Validation of the method was conducted according to ICH Guideline (2005). Calibration curves were prepared in ethanol and linearity was observed within the concentration range of 0.24–50 µg mL^{-1} ($r^2 \geq 0.99$). The limit of detection was 0.49 µg ml^{-1} and the limit of quantification was 1.49 µg ml^{-1}. As previously reported (Parisi et al., 2016) and described by Keizer et al. (2015), the values within LOD and LOQ were treated the same as the values above LOQ, while the measurements below the LOD were considered inaccurate and therefore were noted as not detected.

2.8. Statistical analysis

Data were plotted using Microsoft® Excel (Microsoft, USA) and GraphPad Prism® (Graphpad software, USA). The statistical analysis was carried out using SPSS® Statistics Version 22 (IBM, USA). The results were assessed for normality using the Shapiro-Wilk Test, and the homogeneity of variance was assessed using Levene's test. One-way ANOVA and Independent samples *t*-test were performed where appropriate. Multiple comparison Tukey's HSD post hoc test was used after ANOVA analysis to perform pairwise analysis. For non-normally distributed data or where variances were not equal, the Kruskal-Wallis and Mann-Whitney U Tests and independent sample *t*-test were used, respectively. A p-value lower than 0.05 ($p < 0.05$) was considered statistically significant.

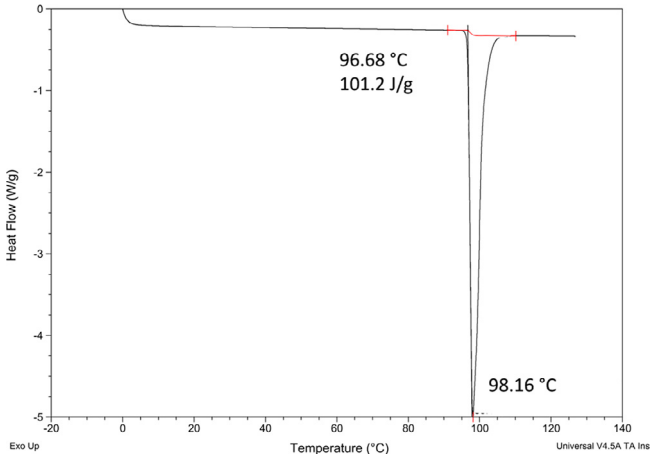

Fig. 2. DSC analysis of CBZ.

3. Results and discussion

3.1. Thermal analysis

The onset temperature of the melting point and the enthalpy of fusion were determined to be 96.68 °C and 29.63 kJ mol^{-1}, respectively as shown in Fig. 2. One single endothermic event in the DSC curve allowed identification of the pure compound, with no impurities or polymorphism evident. Although these results differ from the corresponding values of 98.85 °C and 29.0 kJ mol^{-1} reported for CBZ by Kim et al. (2014), this is likely to reflect the different grades of CBZ available from various suppliers.

3.2. Log $P_{o/w}$, Log $D_{o/w}$ and solubility studies

The predicted value for the Log $P_{o/w}$ of CBZ is 3.01 and experimentally determined distribution coefficients (Log $D_{o/w}$) are reported in Table 1. A significantly lower Log $D_{o/w}$ is evident at more acidic pH values. Since this molecule is a weak base (pK_a = 7.51), the imidazole group will be ionized to a greater extent at lower pH values. Hence, a higher affinity for the aqueous phase is expected, yielding a lower log $D_{o/w}$. In basic solutions (pH values of 7.3 and 9), no differences in the log $D_{o/w}$ were observed (ANOVA, p = 1) but the value was significantly lower at pH 5 (ANOVA, $p < 0.001$).

The solubility of CBZ was evaluated at 32 ± 1 °C and the results are shown in Table 2. The high solubility of the molecule in PG and PEG is consistent with previous reports of the use of these solvents to solubilise azoles (Kovács et al., 2009; Paradkar et al., 2015) and their inclusion in several commercially available solution and gel formulations (Willems et al., 2001). The data also confirm that TC is an excellent cosolvent for CBZ. Despite the lipophilic nature of the molecule, solubility in OSal was comparatively lower than for all other solvents.

Table 1
Log $D_{o/w}$ values of CBZ at pH values 5.0, 7.4 and 9.0. n = 3, mean ± SD. * = $p < 0.01$.

pH	Log $D_{(o/w)}$
5	2.83 ± 0.02*
7.3	3.20 ± 0.03
9	3.20 ± 0.02

Table 2
Solubility of CBZ at 32 ± 1 °C in TC, PEG 200, PG, OSal (n = 4, mean ± SD) and Log Po/w values of TC, PEG 200, PG and OSal extracted from the literature.

Solvent	CBZ solubility (mg ml^{-1})	Log $P_{o/w}$
TC	422.4 ± 4.9	−0.25[†]
PEG	276.3 ± 5.6	−0.93[††]
PG	273.3 ± 8.8	−0.47[†]
OSal	39.0 ± 2.1	5.97[†††]

[†] (Haque et al., 2017).
[††] (Oliveira et al., 2012).
[†††] (Santos et al., 2010).

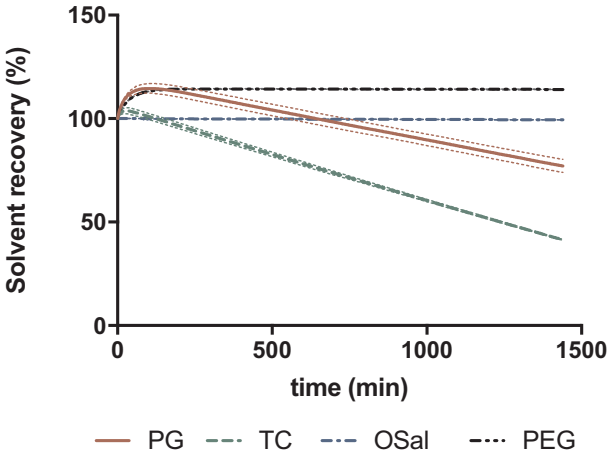

Fig. 3. Percentage of weight loss from 1% CBZ solutions in PG, TC, OSal and PEG as prepared for infinite and finite permeation studies. n = 3, mean ± SD.

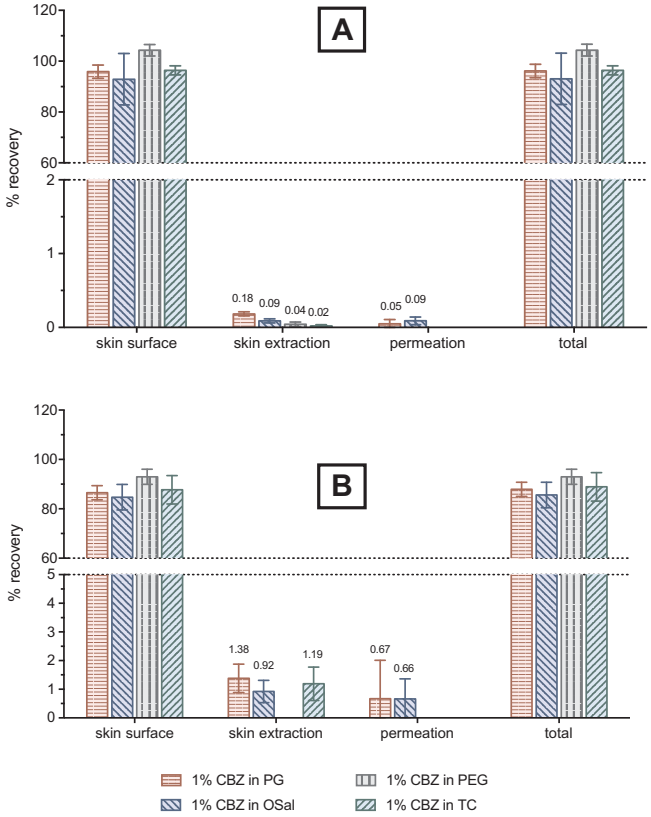

Fig. 4. Percentage of CBZ recovered from applied dose after mass balance in human epidermis for (A) infinite dose application of 1% (w/v) CBZ in PG, OSal, PEG and TC and (B) finite dose application of 1% (w/v) CBZ in PG, OSal, PEG and TC. 4 ≤ n ≤ 7, mean ± SD.

Solubility studies were also conducted in ethanol and DMSO. High CBZ solubility was observed in ethanol (> 0.8 g/ml), in line with published data (Kim et al., 2014). CBZ also showed high solubility values in dimethyl sulfoxide (DMSO), with values > 1.0 g/ml. These results also confirmed the suitability of these solvents for the extraction and surface cleaning procedures in mass balance studies.

3.3. DVS results

Fig. 3 shows the DVS results for all CBZ solutions over the time course of the permeation studies (24 h) at 32 ± 1 °C and 50 ± 2% RH. Although the PG and TC formulations showed an initial increase in weight because of their hygroscopic nature, the solvents evaporated over time, consistent with the evaporation of neat PG and TC reported in our previous study (Haque et al., 2017). No mass changes were evident for the PEG and OSal formulations; to our knowledge DVS has not been used previously to characterise the behaviour of these solvents under controlled conditions of temperature and humidity.

Fig. 4 shows the percentage recovery of CBZ from the skin surface, extracted from the skin and any recovery in the receptor fluid. For both infinite and finite dose applications 1% (w/v) CBZ was applied; the respective volumes were 250 µl and 10 µl for infinite and finite dose experiments. The overall recoveries were within the acceptable range of 80%-120% (OECD, 2004a). Acceptable overall recovery of the active during mass balance studies confirmed that CBZ was stable during the course of the permeation and sample analysis time. The stability of the formulations was also assessed during preformulation studies to ensure no degradation was observed up to 4 days as shown in Fig. A.1 in the tested formulations and PBS.

CBZ acts as an antidandruff agent by preventing the synthesis of ergosterol in fungal cells, acting primarily in the superficial layers of the stratum corneum, where the fungus is mainly located (Piérard-Franchimont et al., 2006). Therefore, an optimal formulation would deliver the active specifically to the skin, minimising transdermal delivery to avoid systemic levels. The key parameter to be considered for the effective topical delivery of CBZ is therefore the amount extracted from the membrane. Three of the four vehicles delivered higher percentages ($p < 0.05$) of CBZ into the skin samples for the finite dose studies (Fig. 4B), when compared with infinite dose studies (Fig. 4A). In absolute amounts, infinite doses of CBZ in PG delivered 4.6 ± 0.6 µg to the membrane compared with 1.5 ± 0.5 µg for finite dose studies, i.e, 0.18 ± 0.03% versus 1.4 ± 0.5% from the applied doses, respectively. Therefore, absolute amounts delivered to the membrane were higher from the infinite doses, but the relative amounts recovered were higher for finite dose studies. Similarly, recovery of CBZ from the OSal vehicle was 2.5 ± 0.8 µg for infinite doses compared with 0.9 ± 0.4 µg for finite doses. For the TC vehicle, the amount of CBZ extracted from the skin was 0.5 ± 0.2 µg for the infinite dose compared with 1.2 ± 0.6 µg for the finite dose studies.

The DVS results indicate that PG and TC will evaporate from the finite dose studies to a much greater extent than the infinite dose studies. Therefore, the thermodynamic activity of CBZ will also change much more rapidly when applied in finite versus infinite doses. The depletion of vehicles because of evaporation has also been reported to have a marked effect on skin permeation and uptake of actives (Selzer et al., 2013). Oliveira et al. (2012) have also reported the use of volatile solvents to increase the permeability of methyl paraben. The surface tension of the solvents in the formulation along with the small amounts applied can hinder the uniform spreading of the preparation on the skin

(van de Sandt et al., 2004). Even though the small volumes were distributed with a plastic inoculating loop, the formulation tended to form droplets on the surface after some time, leading to higher variability observed in the permeation and extraction values characteristic of finite dose studies. These differences have also been reported by Selzer et al. (2013), who highlighted the difficulties in measurements for finite dose studies.

Although infinite dose studies allow the calculation of steady-state permeation parameters, the results reported here confirm that results from such studies may not be extrapolated to finite dose studies that better simulate "real-life" use of topical preparations. As we have noted previously, for prediction of formulation efficiency and selection of appropriate solvents, finite dose studies must be preferred over infinite dose conditions (Lane, 2013).

When the recovered amounts of CBZ in the receptor phase were compared between finite and infinite dose experiments, no statistical differences were found ($p > 0.05$). The relatively low permeation of CBZ for both infinite and finite dose studies is surprising because, as noted above, the molecule has favourable physicochemical properties for dermal penetration. Fig. 4 shows that CBZ only permeated through the skin from the PG and OSal formulations and permeation was evident only after 20 h. The recovered amounts of CBZ were very low and close to the limit of detection. The reasons for the low permeability of CBZ remain to be elucidated, although keratin binding has been reported for other members of the imidazole family including omoconazole, bifonazole and clotrimazole (Hashiguchi et al., 1998).

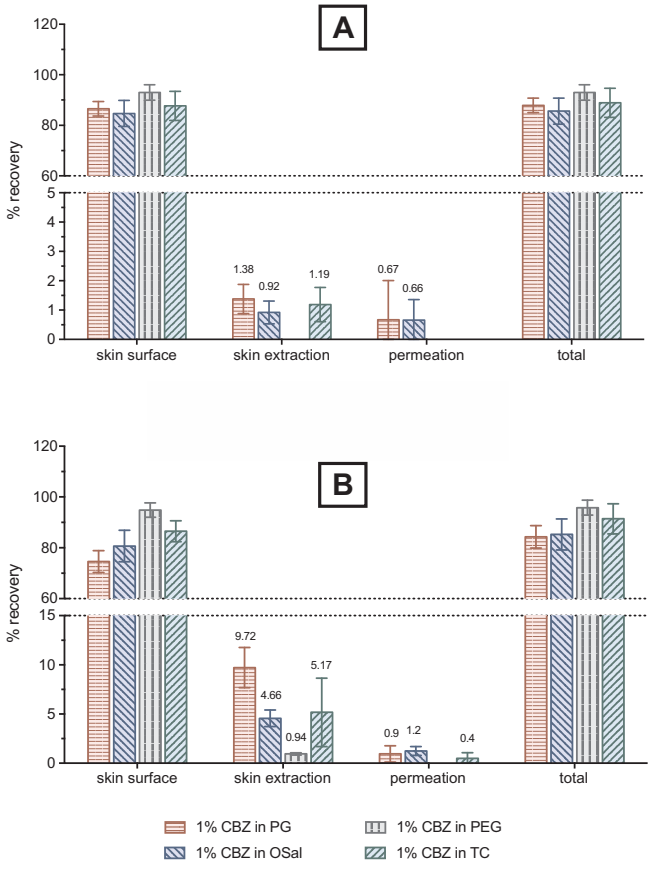

Fig. 5. Percentage of CBZ recovered from applied dose after mass balance in (A) abdominal human skin finite dose application of 1% (w/v) CBZ in PG, OSal, PEG and TC (B) porcine ear skin finite dose application of 1% (w/v) CBZ in PG, OSal, PEG and TC. $4 \leq n \leq 8$, mean ± SD.

3.4. Comparison of permeation and mass balance studies in human skin and porcine skin

Results for the permeation and mass balance studies in human and porcine skin are shown in Fig. 5. The percentage of CBZ extracted from porcine skin was statistically higher for PG ($p = 0.058$), OSal ($p < 0.01$) and TC ($p < 0.01$) compared with human skin, while it was not statistically different for PEG ($p > 0.05$). On the other hand, the permeation values for the applied dose were in all cases not statistically different ($p > 0.05$). Porcine skin is generally regarded as more permeable than human skin, and many comparative studies have been reported in the literature. Many of these have been summarised in a comprehensive review by Barbero and Frasch (2009). These authors reported the permeability of 26 different chemicals in human and pig skin and a correlation coefficient (r) of 0.88 ($p < 0.0001$) was obtained, suggesting that pig skin is an acceptable surrogate for human skin for the compounds evaluated. The factor of difference (FOD) was for 80% of the measurements $0.3 > FOD > 3$, which represents a good prediction. However, in most cases the factor of difference between both surrogates was higher than one, indicating that porcine skin generally yields an over prediction compared with human skin. This observation was also reported by Dick and Scott (1992), Singh et al. (2002) and Luo et al. (2016) for mannitol and paraquat, hydrocarbon structures (heptane, hexadecane, xylene) and ibuprofen, respectively. This further suggests that CBZ may be binding or "stranded" in the skin, despite its suitable properties for percutaneous penetration.

The PG solutions were the most effective formulations in porcine skin ($p < 0.01$) but not in human skin ($p > 0.05$). PG solutions delivered 1.5 ± 0.5 µg of CBZ to human skin compared with 10.9 ± 1.9 µg for porcine skin. PG has been used as a cosolvent and penetration enhancer in a range of topical formulations (Trottet et al., 2004; Duracher et al., 2009; Lane, 2013). Although its enhancing mechanism has not been fully elucidated, it has been suggested that the molecule may intercalate between the polar heads of the lipid lamellae of the *stratum corneum*, increasing the gaps in the lipid bilayers and consequently enabling a higher flux (Brinkmann and Muller-Goymann, 2005; Lane, 2013). TC and OSal delivered approximately half of the relative amounts of CBZ to the skin compared with PG. Both TC and OSal have also been used as penetration enhancers in skin formulations but have been reported to have different mechanisms of action (Lane, 2013). OSal is a lipophilic solvent and the residence time of the solvent in skin is much longer than that for hydrophilic compounds such as PG (Santos et al., 2011). The ability of TC to act as a penetration enhancer was previously ascribed to the faster and greater skin penetration of this molecule compared with other solvents (Haque et al., 2017).

The porcine skin used in this study included epidermal and dermal layers while heat-separated human skin only comprises epidermal tissue. Although this difference in skin thickness may contribute to the higher relative skin extraction values for CBZ in porcine skin, there are conflicting reports in the literature. Vallet et al. (2007) studied the permeation of pesticides in split-thickness and full thickness samples for both human and porcine skin. No statistical differences were observed in the overall amounts of the compounds extracted from the various skin samples. In contrast, there was a statistical difference ($p = 0.04$) in human skin penetration rates compared with porcine skin. Similarly, no differences were observed for the flux values of caffeine following application to dermatomed skin (500 µm) and heat separated epidermis (Atrux-Tallau et al., 2007). Differences in the histological structures of porcine and human skin and the interaction of the active with these membranes should also be considered when accounting for the higher extraction of CBZ from porcine skin compared with human skin. The size of hair follicles in porcine skin has been reported to be nearly twice that in human skin, being 177 ± 4 and 97 ± 3 µm, respectively (Jacobi et al., 2007). Taking into account the favourable conditions for hydrophilic and high molecular weight compounds to permeate across hair follicles (Mitragotri, 2003), the appendageal pathway (including

hair follicles and sweat glands) may explain why both models can uniquely interact according to the chemistry of the permeant. Differences in the molecular structure of both human and porcine skin have also been studied. (Greve et al., 2008) showed by using a combination of near-FT-Raman, FT-NIR and ATR-FTIR, that human skin *in vivo* presented higher lipid content and lower water mass than its porcine counterpart. The lipid content of the SC has also been investigated over the years, showing a different composition in human than porcine skin, more specifically on the ceramides chain length and fatty acid composition (Mojumdar et al., 2014; Wertz et al., 1985). Moreover, the different lipid lamellar structures of human skin compared with porcine skin might also explain the differences observed. It has been reported that human skin has an orthogonal lipid lamellar structure whereas porcine skin lipids have a hexagonal, more fluid lamellar structure (Caussin et al., 2008). A more fluid lipid structure could theoretically lead to higher values of uptake and / or permeation of the active. More recently, (Choe et al., 2018) used Confocal Raman microscopy to study the differences between human and porcine skin. Results showed higher hydrogen bonding of water in human skin, consistent with higher NMF concentrations, as well as a more folded structure of keratin in porcine skin, which could explain a higher permeability of porcine skin.

The permeability of human and porcine skin appears to vary depending on the molecule of interest, and so does the uptake from the membrane. This is clearly the case for CBZ, where statistically higher amounts were extracted from the membrane, but similar values of permeation were reported. As noted, further studies are needed to examine these findings including any possibility of keratin binding or other interaction with the skin.

4. Conclusions

To our knowledge this is the first study that has examined skin uptake and permeation properties of CBZ in porcine and human skin. Even though CBZ has appropriate physicochemical properties for dermal penetration, no permeation was observed for some vehicles and where CBZ could be detected in the receptor compartment, the amounts recovered were very low. This is surprising when the physicochemical properties of CBZ are compared with those of ibuprofen, a known skin permeant. Both actives have molecular weights < 300 Da, melting points < 100 °C and Log P and Log D values that favour percutaneous delivery. As reported for other antifungal imidazoles, this may reflect an interaction of CBZ with the keratin of the *stratum corneum*. Skin retention of CBZ rather than penetration is desirable considering the intended use of the active; nevertheless, the underlying reasons for the preferential deposition of CBZ in the outer skin layers deserve further investigation.

Skin delivery of CBZ was examined from four solvents under finite and infinite dose conditions. The results confirm, once again, the need to interpret findings from infinite dose experiments with caution when developing topical formulations of actives. For infinite dose studies lower amounts of CBZ were recovered following skin extraction, when compared to finite dose studies. DVS studies on the various formulations also simulated the “drying down” or metamorphosis that occurs following application of finite doses of topical preparations to the skin and have also not been reported previously for CBZ. PG and OSal appear promising candidate vehicles for CBZ and should be considered as components of more complex formulations or binary systems.

Although porcine ear skin is widely accepted as a useful surrogate for human skin, significantly higher amounts of CBZ were extracted from the former compared with the latter. This likely reflects differences in morphology and composition of the tissues but again also points to specific interactions of CBZ with skin that vary depending on the membrane selected for study. Future work will expand the range of vehicles examined using finite dose studies in human skin with the ultimate objective of achieving concentrations that approximate the MIC required for *Malassezia spp* in the skin. More permeation studies focusing on the penetration of solvents, as well as keratin binding studies may also shed light to the reason behind the low permeability of CBZ.

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Appendix

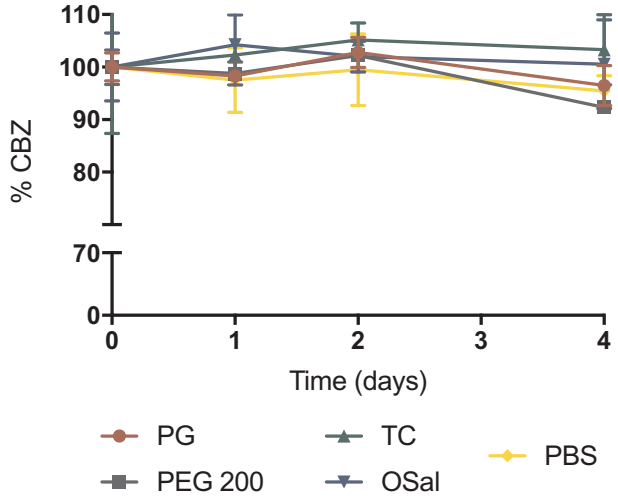

Fig. A.1. Stability of 1% CBZ formulations in PG, PEG 200, TC, OSal and 0.01% in PBS over a 4 day period. n = 3, mean ± SD.

Table A.1
Percentage of CBZ recovered in the surface, skin extraction, permeation and total recovery from applied dose for 1% (w/v) CBZ formulations in PG, PEG, TC and OSal tested in human abdominal skin using infinite and finite doses; and porcine skin using finite doses. $4 \leq n \leq 5$, mean ± SD.

		Surface	Extraction	Permeation	Total recovery
PG	Infinite human	95.9 ± 2.6	0.2 ± 0.03	0.05 ± 0.06	96.1 ± 2.6
	Finite human	86.5 ± 2.8	1.4 ± 0.5	0.7 ± 1.3	87.9 ± 2.9
	Finite pig	74.6 ± 4.3	9.7 ± 2.0	1.0 ± 0.8	84.3 ± 4.4
PEG 200	Infinite human	104.3 ± 2.6	0.04 ± 0.03	0.0	104.3 ± 2.3
	Finite human	93.0 ± 3.0	0.0	0.0	93.0 ± 3.0
	Finite pig	94.9 ± 2.8	0.9 ± 0.1	0.0	95.8 ± 2.9
TC	Infinite human	96.4 ± 1.8	0.02 ± 0.02	0.0	96.4 ± 1.8
	Finite human	87.7 ± 5.8	1.2 ± 0.6	0.0	88.9 ± 5.7
	Finite pig	86.5 ± 4.1	5.2 ± 3.5	0.5 ± 0.6	91.4 ± 5.9
OSal	Infinite human	92.9 ± 10.1	0.09 ± 0.03	0.09 ± 0.05	93.0 ± 10.1
	Finite human	84.7 ± 5.1	0.9 ± 0.4	0.7 ± 0.7	85.6 ± 5.2
	Finite pig	80.7 ± 6.2	4.6 ± 0.8	1.2 ± 0.4	85.3 ± 6.1

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