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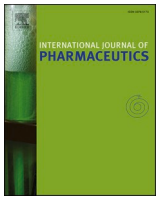

Rational development of topical climbazole formulations

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ABSTRACT

Dandruff, or *pityriasis capitis simplex*, is a common scalp condition associated with excessive flaking and scaling of the epidermal tissue. Other features include irregular corneocyte turnover, irritation, itching and an impaired skin barrier function. Previously we reported the characterization of climbazole (CBZ), an antifungal agent used in the management of dandruff. Skin permeation of CBZ from neat solvents was also investigated. In the present work we evaluated CBZ permeation in human skin *in vitro* from more complex formulations that better represent products used by consumers. The various systems studied were composed of propylene glycol (PG), Transcutol®P (TC), octyl salicylate (OSal) and isopropyl alcohol (IPA). As well as measurement of skin uptake and penetration of CBZ, where possible, the skin retention and permeation of the various solvents was also determined. All vehicles promoted skin permeation of CBZ but no significant differences in amount permeated were evident between the binary vehicles (PG:TC, TC:OSal) and the ternary vehicle studied (PG:IPA:OSal). The binary vehicles generally promoted more skin uptake of CBZ compared with the neat solvents (PG, TC, OSal) studied previously. Permeation and skin extraction of CBZ from the PG:TC vehicles increased with increasing PG content; a similar trend was evident for the PG:IPA:OSal systems. New methods were developed and validated for measurement of PG, TC and OSal. Analysis of the individual solvents indicated that PG permeation was also independent of the amounts of other solvents in the binary or ternary systems. Consistent with previous findings higher proportions of TC permeated compared with PG for the PG:TC binary systems; TC also permeated the skin more rapidly than PG from these vehicles. For OSal, skin extraction was generally higher for TC:OSal compared with the PG:IPA:OSal vehicle. However, increasing the content of OSal did not appear to influence CBZ skin uptake nor permeation. Interestingly, the effects of the various PG:TC vehicles on CBZ skin delivery contrast with results we previous reported for the same systems for a different active. This confirms that with reference to skin permeation, formulation effects and/or skin penetration enhancement should be expected to vary and may not be predicted for specific vehicles.

1. Introduction

Formulation development comprises a considerable part of a product's lifecycle before commercialisation. Initially, the physicochemical properties are characterised during pre-formulation studies to ensure the stability and suitability of simple formulations. The next stage of the programme typically involves *in vitro* studies, and for topical formulations this allows the appropriate parameters for skin penetration studies are established. These include surrogate and sampling protocol selection with the objective of reproducing real life conditions as closely as possible (Flaten et al., 2015). In vitro studies are also key to identify the penetration profile of an active, that will be formulated differently according to the expected site of action. Once the foundations of the formulation have been laid, further studies follow to obtain the desired outcomes for the formulation, i.e., topical or transdermal delivery, leave on or rinse off application.

We previously reported the characterization of the anti-dandruff active, climbazole (CBZ) and the skin permeation of the compound from simple solvents (Paz-Alvarez et al., 2018). The findings confirmed that CBZ was largely delivered to the outer layers of the skin for the simple solvents studied. However, the physicochemical properties of CBZ indicate that the compound should be suitable for delivery through the skin, not just to the skin. Typically, CBZ is found in shampoos and products applied to the scalp; the scalp is also more permeable than the skin (Rolland et al., 2011). Recently, regulation EU 2019/698 also noted that there is a potential risk to human health arising from the use of CBZ

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as a preservative or as a non-preservative in cosmetic products (EU 2019/698, 2019). Thus, the ability of the molecule to access the deeper layers of the skin is of considerable interest to regulators and scientists in the personal care sector. Therefore, in the present study our aim was to investigate how different formulation-based approaches may affect local delivery versus percutaneous penetration of CBZ. Lane et al. (2012) highlighted the relevance of thermodynamic activity and partition coefficient in topical formulations. Both parameters are driven by the formulation, so finding appropriate vehicles is critical for the development of functional formulations able to deliver the active(s) efficiently and safely. Consequently, in the present work particular attention was paid to the role of solvents in the formulation.

Initially, solvents were prepared as binary and ternary systems to evaluate their efficacy for delivering CBZ to the skin. The residence or permeation of solvents in the membrane may also contribute to penetration enhancement, and where possible, this was also investigated. Two binary systems and one ternary system were prepared from propylene glycol (PG), Transcutol P (TC), octyl salicylate (OSal) and isopropyl alcohol (IPA). These solvents were chosen based on the results from our previous work with PG, TC and OSal. IPA was necessary in to ensure the immiscible PG and OSal could be included in the ternary system. Specific ratios of solvents were selected based on miscibility determinations and/or where we have investigated the same combinations of these solvents for *in vitro* skin delivery of other actives.

2. Materials and methods

2.1. Materials

Climbazole (Crinipan® AD, Symrise, Germany) was supplied by Unilever (Port Sunlight, UK). Propylene glycol (PG), 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 Sigma Aldrich, UK. Abdominal human skin was obtained following plastic surgery from a single female donor with institutional ethical approval and informed consent, provided by a licensed tissue bank (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. CBZ formulation preparation and miscibility studies

All formulations (Table 1) were freshly prepared before analysis as required in 1 ml quantities. The appropriate mass of CBZ was weighed into a screw cap glass vial and the solvents were added to give a final concentration of 1 % (w/v) CBZ. All measurements were conducted using an analytical balance, accurate to 0.0001 g (Mettler Toledo, USA).

Miscibility studies of ternary solvent systems were conducted for the mixtures of PG, OSal and IPA (Merck, Germany). Sudan III (Sigma Aldrich, UK) was added as a hydrophobic marker of miscibility. Approximately 5 mg of Sudan III was added to microcentrifuge tubes and dissolved by adding different amounts of OSal. Subsequently, PG and IPA were added at different ratios, the tubes were shaken for approximately 1 min and miscibility was assessed visually. The composition of binary and ternary systems is described in Table 1. Ternary phase diagrams (data not shown) were plotted using OriginPro 9.0 software (Originlab®, USA).

Table 1
Binary and ternary phase systems examined in this work containing climbazole (CBZ), propylene glycol (PG), octyl salicylate (OSal), Transcutol P (TC) and isopropyl alcohol (IPA).

% Formulation		CBZ	PG	OSal	TC	IPA
PG:TC	75:25	1	74.25	-	24.75	-
	50:50	1	49.5	–	49.5	–
	25:75	1	24.75	–	74.25	–
	45:15:40	1	44.55	14.85	–	39.6
PG:OSal:IPA	30:30:40	1	29.7	29.7	–	39.6
	15:45:40	1	14.85	44.55	–	39.6
	75:25	1	–	74.25	24.75	–
OSal:TC	50:50	1	–	49.5	49.5	–
	25:75	1	–	24.75	74.25	–

2.3. High performance liquid chromatography (HPLC) analysis of CBZ

A HPLC method was developed and validated for CBZ as reported previously (Paz-Alvarez et al., 2018). Briefly, 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.

2.4. Gas chromatography (GC) analysis of PG, TC and OSal

A 7890A GC system (Agilent Technologies, USA) equipped with a flame ionisation detector (FID) was employed for GC analysis, using a diphenyl dimethyl polysiloxane column HP-5 (Agilent, USA) with a length, internal diameter and film thickness of 30 m, 0.32 mm and 0.25 µm, respectively. The injection volume was 0.5 µl with a 0.2 µl air gap at 1:1 split ratio with 2 ml min^{-1} septum purge flow. Nitrogen was used as carrier gas at constant flow of 2 ml min^{-1}. The inlet temperature was programmed at 225 °C, followed by 1 min equilibration time. The oven temperature was initially set at 80 °C, increasing at 7 °C min^{-1} until 120 °C and from there at 20 °C min^{-1} until reaching 200 °C. The FID temperature was set to 325 °C and the flame was ignited using hydrogen, air and nitrogen as make-up gas at 35, 350 and 30 ml min^{-1}, respectively. The retention times were 2.7 min for PG and 4.6 min for TC. These peaks were used for quantification by interpolation from the calibration curve after validation of the method.

The analytical method for TC and OSal binary systems used the same equipment and conditions. The only change was the oven temperature, which was initially programmed at 70 °C and equilibrated for 1 min. The temperature was increased at 30 °C min^{-1} to 110 °C, from there to 200 °C at a rate of 20 °C min^{-1} and left at that temperature for 1 min. Ignition gas flow and temperatures for the FID remained the same as in previously described methods. The retention time for TC was 3 min and 5.4 min for OSal; these were used for quantification after validation of the method.

The analytical method for quantitation of PG and OSal systems was similar with some modifications. Injection volume and air gap were maintained, while the inlet temperature was reduced to 200 °C. Total gas flow was set to 1.8 ml min^{-1} during the run and at 2 ml min^{-1} for the post run purge. The initial temperature for the oven was set to 50 °C after 1 min equilibration time and increased at a rate of 30 °C min^{-1} to 110 °C. It was then increased at 45 °C min^{-1} from 110° to 300 °C and held for 0.5 min at that temperature. Peaks eluted at 3.5 min for PG and 6.1 min for OSal, which were used for quantification after validation of the method.

2.5. Finite dose permeation studies

Approximately 10 µl cm^{-2} of the formulation were applied using a positive displacement pipette (Eppendorf®, UK). 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 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 regular intervals over the duration of the permeation 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. Data analysis

Data were plotted and statistical analysis was carried out using GraphPad Prism® (Graphpad software, USA). Where cells leaked the data were discarded and not used in the analysis. 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.

3. Results and discussion

PG, OSal, TC and IPA were incorporated into binary and ternary systems at different ratios, to previously, the ideal formulation would increase the delivery of active to the skin while minimising permeation. The analysis of solvents was also conducted in order to elucidate potential mechanisms of delivery. Mass balance studies were conducted not only for CBZ, but also, where possible, for the solvents using GC, which allowed their quantification and investigation of any correlation with the amounts of extracted CBZ.

3.1. Permeation and mass balance studies of CBZ from PG:TC systems

PG and TC were combined at three different ratios and the results for mass balance studies are shown in Fig. 1(A). For all PG:TC vehicles most of the active (>70 %) remained on the skin surface; these values were statistically different from results for total recovery ($p < 0.05$). Values for total recovery were also in line with OECD guidelines (OECD, 2011). The combination of TC with PG promotes CBZ skin uptake compared with either PG or TC alone (Paz-Alvarez et al., 2018). For example, for the PG:TC (75:25) system, a 6-fold increase in the extracted amounts (8.4 ± 3.2 % of the applied dose) was achieved when compared to PG alone (Paz-Alvarez et al., 2018). Similarly, a 7-fold increase in CBZ was delivered to the skin for the PG:TC (75:25) system compared with neat TC. When the levels of CBZ extraction were compared, a statistical difference was found between the PG:TC (75:25) and PG:TC (25:75) ratios ($p < 0.05$). This suggests that a higher proportion of PG in the formulation may drive a higher amount of CBZ into the membrane; this is discussed further in the section where PG permeation is reported.

Clearly in the present work, combining PG with TC does promote permeation of the active (Fig. 1(B)). In our previous study no CBZ permeated from TC alone (Paz-Alvarez et al., 2018). The values for absolute permeation of CBZ from the PG:TC vehicles are significantly higher than for the neat PG ($p < 0.05$) reported previously (Paz-Alvarez et al., 2018). In the previous work although permeation of CBZ was

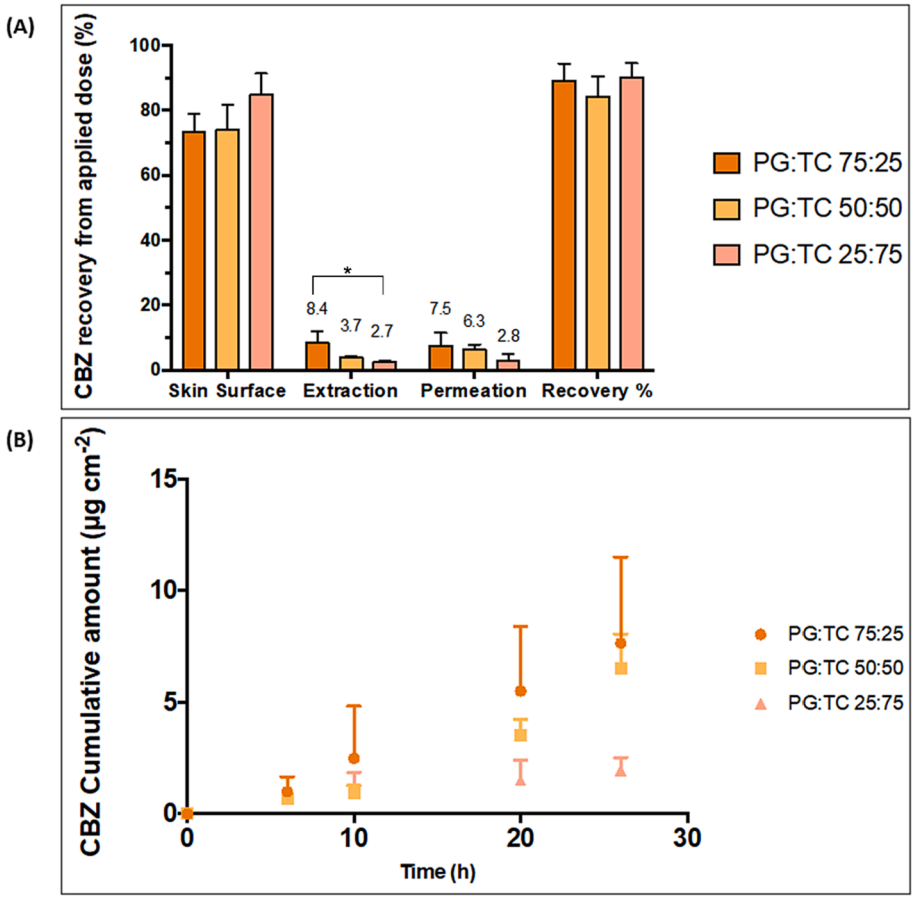

Fig. 1. (A) Total recovery of CBZ from PG:TC binary systems following finite dose *in vitro* studies with human skin, (B) Cumulative permeation of CBZ from PG:TC systems following finite dose *in vitro* studies with human skin. 5≥n≥3, mean ± SD. Asterisk indicates statistical difference ($p<0.05$).

observed for neat PG, this was not evident until the 20 h sampling time point. Very low values for percentage permeation (0.67 %) from neat PG were also observed that were close to the limit of detection for CBZ. Again, as for delivery to the skin, the PG content appears to influence the extent of permeation of CBZ. Interestingly, this is in contrast to the findings of Kung et al. (2020) who examined the same binary PG:TC systems in finite dose studies in porcine skin for methadone. In this work, the highest drug permeation was observed for PG:TC (25:75) and PG:TC (50:50). Although, the drug loading was higher in this earlier study (5 % w/v) the findings also confirm that it is not possible to extrapolate vehicle effects on skin permeation from one molecule to another.

3.2. Permeation and mass balance studies of CBZ from TC:OSal systems

Fig. 2(A) shows the percentage values of dose applied of CBZ remaining on the skin surface, extracted from the skin and percentage permeated for the TC:OSal binary formulations, following *in vitro* permeation studies. As for the binary PG:TC systems, when compared with the neat solvents TC and OSal (Paz-Alvarez et al., 2018), a significant improvement in skin uptake was observed for all TC:OSal vehicles ($p < 0.05$). Overall, a higher percentage of CBZ was recovered from the receptor compartment than from the skin extraction studies ($p < 0.05$). Although higher permeation than extraction is typically the goal of conventional permeation studies, for this active targeted skin retention rather than permeation is the ultimate objective.

The permeation profiles of CBZ for the TC:Osal binary systems are shown in Fig. 3(B); values of 2.9 ± 1.1 μg cm^{-2} were attained at 6 h for TC:OSal (75:25). This is more than twice the amount of CBZ that permeated from the PG:TC vehicles for the same timepoint (Fig. 1(B)). For early timepoints, as shown in Fig. 2(B), no differences in cumulative permeation were evident between the three TC:OSal systems. At the last sampling timepoint TC:OSal (75:25) was observed to deliver the highest amount of CBZ across the skin ($p < 0.05$). Previously, CBZ permeated from either TC or OSal only after 20 h (Paz-Alvarez et al., 2018).

OSal has traditionally been used as a UV filter but has also been reported to facilitate transdermal enhancement by increasing the residence time of actives in solution in the skin (Santos et al., 2012). OSal was reported to permeate the skin slowly (less than 1.5 % permeation over 48 h) with > 14 % of the applied dose accumulating in the skin (Walters et al., 1997). On the other hand, TC has been reported to permeate the skin *in vitro* to a much greater extent than other solvents. Haque et al. (2017) reported that approximately 60 % of the applied amount of TC was able to permeate across human skin within 20 h of application of a finite dose of anthramycin in TC to human skin. The combination of TC and OSal in binary systems for delivery of actives has not been reported previously to our knowledge.

3.3. Permeation and mass balance studies of CBZ for PG:Osal:IPA systems

The results observed for mass balance studies after application of the ternary system PG:OSal:IPA at three different ratios are shown in Fig. 3 (A). As for the TC:OSal systems, a significant improvement in skin uptake and permeation was observed compared with the individual solvent systems. For PG:Osal:IPA (45:15:40), about 60 % of the applied dose was recovered from the skin surface. However, total recovery results were within the acceptable ranges for all three vehicles (OECD, 2011). Similar amounts of CBZ were retained in the skin also for these ternary systems with no observed statistical differences ($p > 0.05$).

The use of IPA in the ternary formulation is necessary for miscibility of PG and OSal. However, IPA may also contribute to penetration

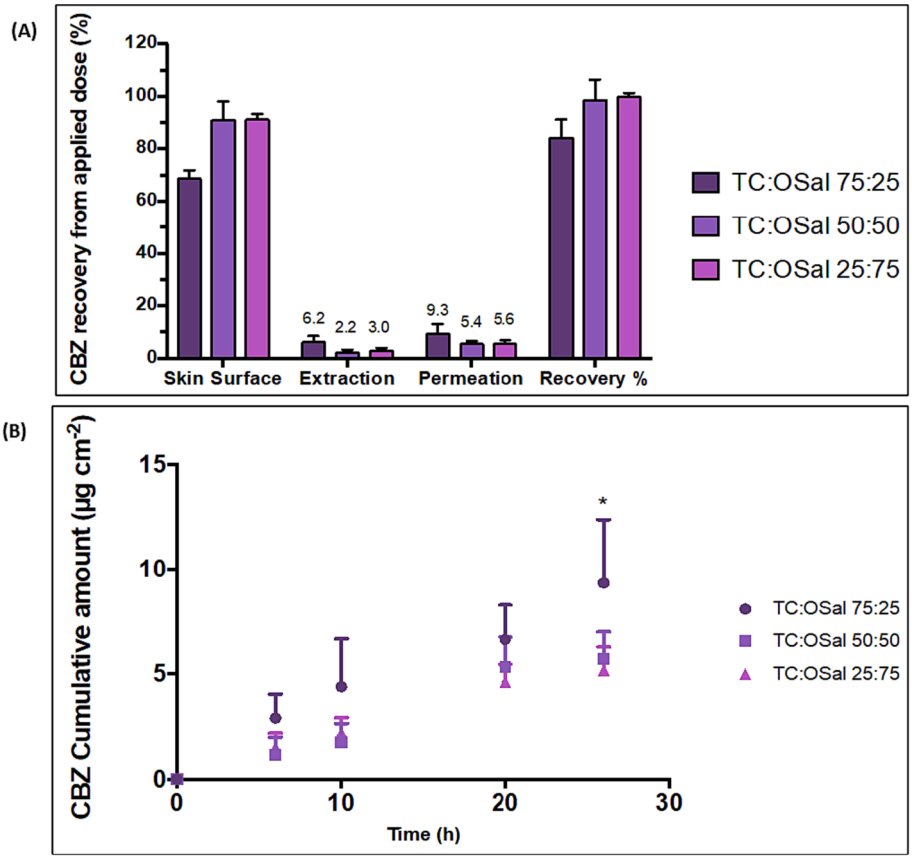

Fig. 2. (A) Total recovery of CBZ from TC:OSal systems following finite dose *in vitro* studies with human skin (B) Cumulative permeation of CBZ from TC:OSal systems following finite dose *in vitro* studies with human skin. $5 \geq n \geq 3$, mean ± SD. Asterisk indicates statistical difference ($p<0.05$).

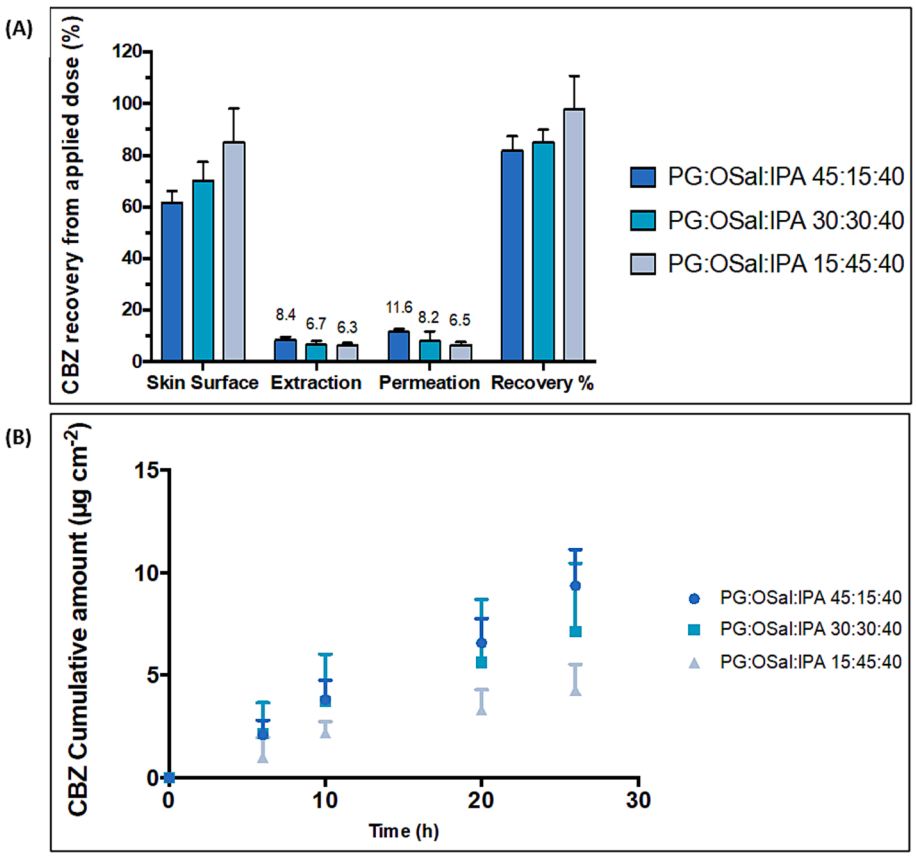

Fig. 3. (A) Total recovery of CBZ for PG:OSal:IPA systems following finite dose *in vitro* studies with human skin. (B) Cumulative permeation of CBZ from PG:OSal:IPA systems following finite dose studies with human skin G:OSal:IPA. 5≥n≥3, mean ± SD.

enhancement. More than 30 years ago, the effect of primary alcohols on transdermal delivery was studied. Noriko et al., (1988) investigated the enhanced delivery of indomethacin and reported a higher enhancement for medium-chain alcohols (C12-C14). Later, Lane (2013) reviewed the permeation enhancement activity of lower chain alcohols and reported some of the mechanisms of penetration enhancement proposed for these compounds. Alcohols are able to increase the solubility of drugs in the vehicle and their permeation can modify the solubility properties of the SC, leading to better active partitioning in the membrane. Additionally, the high volatility of short alcohols would be expected to modify the thermodynamic activity of the active in the formulation, thus promoting skin delivery.

The cumulative amounts permeated of CBZ are shown in Fig. 3(B). No statistical differences were observed among the tested ternary formulations ($p > 0.05$), but if the permeation profiles are fitted linearly all three formulations show a steady state from 6 h ($r^2 > 0.9$). If the slope is calculated from the linear fitting, a higher slope can be clearly observed for the 45:15:40 ratio formulation, suggesting that higher amounts of PG provide a faster permeation of CBZ.

4. Analysis of PG, TC and OSal

Because of analytical limitations the extraction of PG and TC from the membrane could not be measured. Interference with the validated extraction solution composed of DMSO and MeOH, prevented accurate quantification of the solvents in the skin, therefore total mass balance for PG and TC could not be completed. The high concentration of DMSO resulted in a wide peak extending along the chromatogram overlapping with the peaks of PG and TC. Dilution did not resolve the issue as the concentration of analyte was reduced below the LOD.

4.1. Permeation of PG from PG:TC and PG:OSal:IPA vehicles

The permeation of PG and TC across human skin was evaluated for all binary and ternary vehicles. Given the different proportions of PG used for each ratio (Table 1), the permeated amounts may vary accordingly. For all vehicles, PG showed a steady state flux from 6 to 20 h, showing a linear penetration with time as shown in Fig. 4(A). At the end of the experiment, the PG:TC (75:25) formulation delivered 3.7 ± 0.5 mg of PG per cm^2, the highest for all formulations ($p < 0.05$), followed by PG:TC (50:50) and PG:OSal:IPA (45:15:40) with values of 2.1 ± 0.5 mg cm^{-2} and 1.6 ± 0.1 mg cm^{-2} respectively. These results follow the same order as the proportion of PG in the formulations. However, if the cumulative amounts permeated are normalised to the applied amount of PG as a function of time as shown in Fig. 4(B), comparable proportions of PG (approximately 40 %) reached the receptor phase for all formulations. This indicates that the normalised flux of PG was not affected by the addition of increasing amounts of TC for the permeation, whereas the penetration of PG is directly proportional to the applied amounts of PG in absolute terms. With reference to the PG:OSal:IPA systems, the proportion of PG in the formulation was reduced to allow the addition of IPA as a cosolvent. Interestingly, Fig. 4(B) confirms that no statistical differences were found in the permeated amounts of PG between PG:TC (25:75) and PG:OSal:IPA (30:30:40), again indicating that the permeation of PG may be independent of the addition of other solvents.

Haque et al. (2018) also investigated the effect of solvents in the delivery of anthramycin for the topical treatment of skin cancer. The penetration of the active as well as PG was reported for binary systems of PG and propylene glycol monolaurate (PGML), propylene glycol monocaprylate (PGMC) and water as well as for PG alone. For PG:PGML (90:10), more than 70 % of the PG was detected in the receptor phase

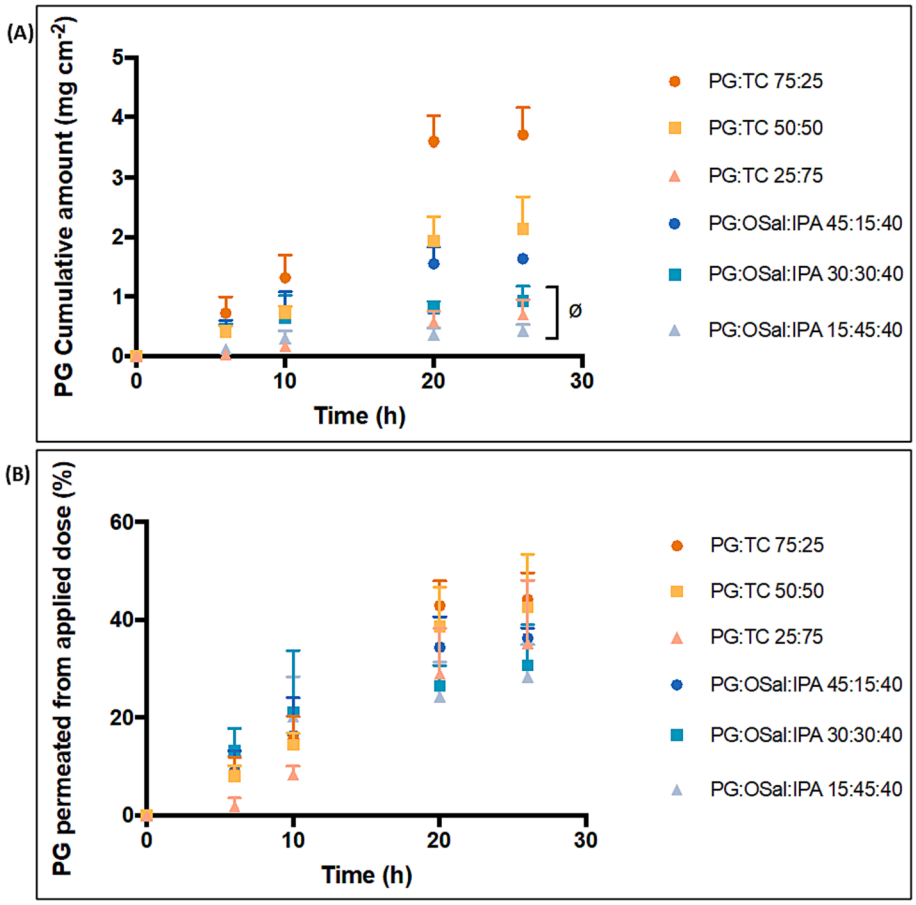

Fig. 4. (A) Cumulative amount of PG permeated per cm^2 after finite dose application of binary and ternary CBZ systems to human skin. $5 \geq n \geq 3$, mean ± SD. Ø indicates no statistical difference ($p > 0.05$). (B) Percentage of PG permeated from applied dose. $5 \geq n \geq 3$, mean ± SD. Asterisk indicates statistical difference ($p < 0.05$).

and corresponding values for PG:PGMC (90:10) and PG alone were 52.1 % and 21.9 % respectively. In an earlier study by Oliveira et al. (2012), the relationship between permeation of methyl paraben and solvent uptake was reported for a model membrane. The authors suggested that solvent uptake by the membrane was closely associated with higher amounts of active in the membrane as well as greater permeation. Considering the results reported here for CBZ, the rank orders for values of CBZ extraction and permeation are consistent with the amounts of PG in the vehicles i.e. higher PG levels are associated with higher CBZ skin retention and permeation (Fig. 1(A).

4.2. Permeation of TC from PG:TC and TC:OSal vehicles

The cumulative amounts of TC that permeated for the various vehicles are shown in Fig. 5(A). As for the results obtained from PG, for the TC formulations with equal proportions of solvent, e.g. PG:TC (75:25) and TC:OSal (25:75), or PG:TC (25:75) and TC:OSal (75:25), comparable amounts of TC had permeated at the last time point ($p > 0.05$), suggesting that the addition of PG or OSal did not significantly affect TC permeation. Comparing Fig. 4(B) and fig. 5(B), a higher proportion of TC permeated when compared to PG. If the cumulative amount of TC permeated is normalised with the applied amount, more than 50 % was recovered in the receptor for most of the formulations, while lower proportions were observed for PG. The PG:TC (25:75) formulation showed the highest recovery in the receptor phase with 74.8 ± 6 % of the applied amount of TC. Another notable difference regarding the permeation of TC is the onset of the permeation. While for PG the amounts recovered in the first 6 h of the permeation were all below 1 mg cm^{-2}, the amount of TC that permeated over the same period was 2.2 ± 0.6 mg cm^{-2} for TC:OSal (50:50). A faster onset of the permeation of TC was characteristic of the TC:OSal formulations (72:25 and 50:50), where higher amounts permeated at early time points of the experiment.

Higher permeability for TC compared to PG was reported by Haque et al. (2017), for saturated anthramycin solutions. More than 50 % of TC was recovered in the receptor compartment compared to approximately 30 % recovery for PG. More recently Patel et al (2021) investigated the delivery of ibuprofen from a series of solvents under finite dose conditions. The percentage of PG that permeated was approximately 20 % of the initial dose. This might reflect the lower dose applied (3.6 µl/cm^2) and the higher drug loading compared with the present work (5 % w/v ibuprofen). Depletion of PG during the permeation study was also evident.

4.3. Permeation of OSal from TC:OSal and PG:IPA:OSal vehicles

The mass balance studies for OSal are shown in Fig. 6. The higher boiling point of OSal allowed the measurement of the extraction using GC as the relevant peak did not overlap with the DMSO peak. No OSal was detected in the receptor solution at the end of the permeation experiment for any of the formulations. This indicates that OSal remained on the surface as well as inside the skin. Given the hydrophilic nature of the viable epidermis, a molecule with a comparatively higher log $P_{o/w}$ like OSal would be expected to present low permeation, which is confirmed by the experimental data. However, the *in vitro* experiments had been primarily designed and validated for the study of CBZ. Hence, the receptor solution was composed of PBS only. As OSal is a highly lipophilic solvent, besides limited permeation, low partition in aqueous systems would be expected, and therefore sink conditions may have not

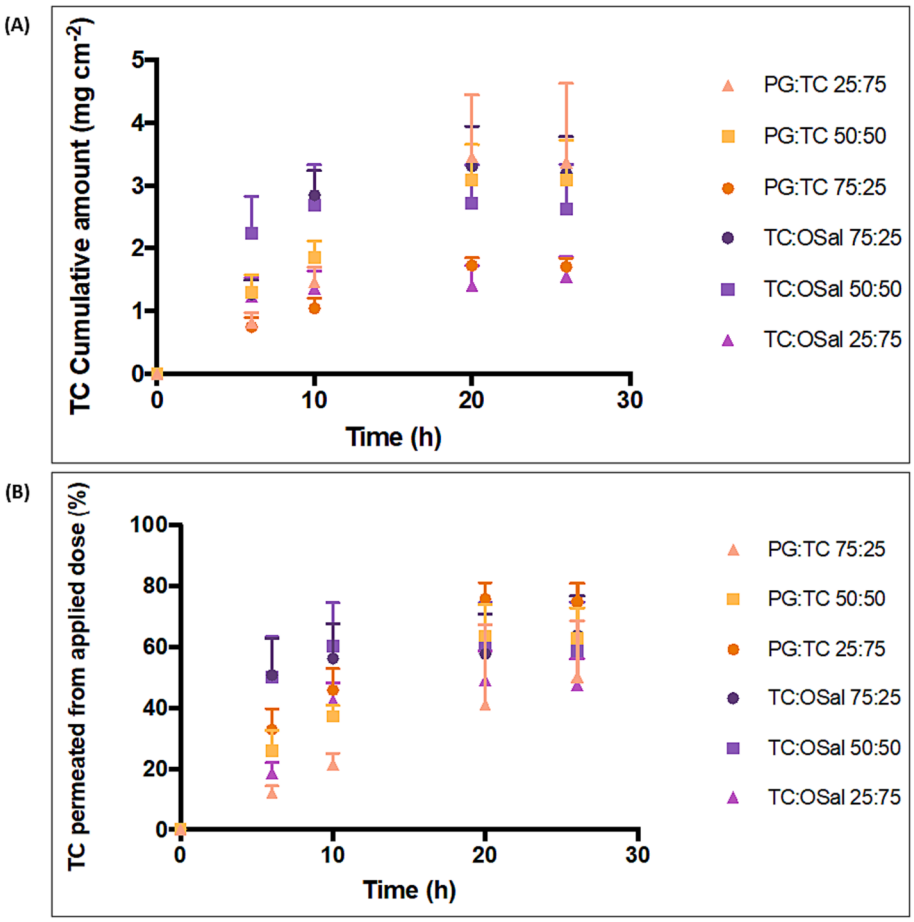

Fig. 5. (A) Cumulative amount of TC permeated per cm^2 following finite dose application of binary and ternary CBZ systems to human skin (B) Percentage of TC permeated from applied dose. $5 \geq n \geq 3$, mean ± SD.

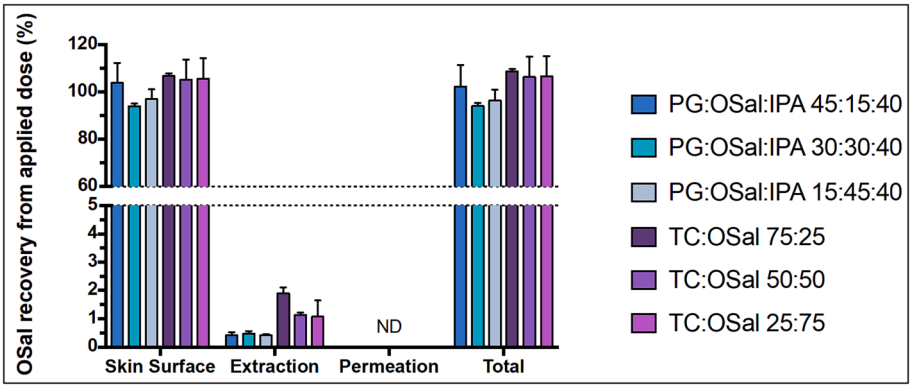

Fig. 6. OSal mass balance results following application of binary and ternary CBZ systems as finite doses to human skin. $5 \geq n \geq 3$, mean ± SD.

been achieved for OSal. The skin permeation of OSal was studied previously by Santos et al. (2010;2012), who reported low but steady permeation of OSal across human epidermis. The main difference in that study was the inclusion of 0.5 % (w/v) PEG-20-OE as a solubiliser in the receptor media, to increase the solubility of OSal in the receptor solution for sink conditions for OSal. The absolute values for permeation were of the order of nmol cm^{-2} and independent of the concentration of OSal in the formulation. Similar permeation results were reported by Walters et al. (1997), but again a solubiliser (6 % w/v Volpo N20) had been added to the receptor solution.

An earlier study reported low total recoveries of OSal in the range of 60–70 % of the applied amount (Santos et al., 2010). These results contrast with the higher recoveries showed for the TC:OSal and PG:OSal:IPA solutions reported here which all were above 80 % (Fig. 6). However, OSal was prepared as a spray formulation with ethanol in the previous work and this may explain the recovery values. The doses were also lower than those used in the present work (3.6 µl versus 10 µl). The findings here do however support the conclusions of Santos et al (2010) concerning the role of OSal in topical/transdermal formulations; permeation of OSal may not be the most important feature for its enhancement of skin penetration of actives. Instead, the ability of this solvent to remain on/in the skin to provide a longer residence time of the

actives appears to be important for effective topical/transdermal delivery of molecules.

5. Conclusions

In previous work the permeation profile of CBZ was evaluated for single solvent systems. Observed values of extraction were lower than 2 % in human skin; permeation was also only observed towards the end of the permeation experiment (after 20 h). In the present work more complex formulations were prepared with the aim of increasing the delivery of the active to the skin. From the solvents studied previously as single solvents, the highest CBZ skin extraction values had been observed for PG, OSal and TC, which led to combinations of these into binary and ternary systems.

The results confirmed significant increases in percentage skin extraction values for CBZ when compared to single solvent systems. Values increased up to 6-fold for PG:OSal:IPA (45:15:40) and PG:TC (75:25), values that are greater than the individual contribution of each solvent combined. This means synergy between the solvents was achieved to increase the skin uptake. However, no statistical differences for values of skin uptake were observed among different solvent combinations. The permeation of solvents was also evaluated, and in line with previous reports, TC showed a higher transdermal flux than PG, while no permeation was observed for OSal.

A higher penetration of PG was associated with a higher extraction of CBZ for the PG:TC and PG:OSal:IPA systems independently. The permeation of PG also appeared to drive the delivery of CBZ to the skin. Therefore, by studying the penetration enhancement of PG, the development of more efficient CBZ formulations may be approached from a different angle i.e. modulating the amounts of PG in formulations may allow enhanced uptake but skin penetration needs to be minimised. This might be achieved by exploring a wider range of formulation components. In contrast, there was no evident trend for OSal content in the binary or ternary vehicles; for the ternary vehicle increasing OSal content was associated with lower skin extraction and permeation.

As observed in previous work, TC penetrated to a greater extent than PG. Additionally, the penetration of PG and TC was not significantly altered by the addition of increasing amounts of a second solvent in complex systems. OSal was mainly recovered from the skin surface (>90 %) and smaller amounts were also recovered from skin extraction studies. Finally, the findings observed here for the PG:TC vehicles contrast with previous studies we have reported for the same vehicles for methadone. This confirms that vehicles effects observed for one active may not be expected for a different active, underlying the complexity of the interplay between vehicle, active and the skin.

CRediT authorship contribution statement

Miguel Paz-Alvarez: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Chun Fung Tang:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Paul D. Pudney:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Majella E. Lane:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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