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Investigation of binary and ternary solvent systems for dermal delivery of methadone

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Abstract

Methadone appears to be a promising candidate for pain management. Previously, we conducted a comprehensive characterization study of methadone base and evaluated the dermal delivery of methadone from various neat solvents. Four solvents, namely d-limonene (LIM), ethyl oleate (EO), Transcutol® P (TC) and octyl salicylate (OSAL), were identified as the optimal neat solvents for skin delivery of the compound. To explore further approaches to improve methadone permeation, the present work investigated a range of binary and ternary vehicles. *In vitro* permeation studies in porcine skin confirmed that binary systems delivered significantly higher ($p < 0.05$) amounts of methadone through the skin compared with neat solvents. The highest skin permeation was observed for formulations composed of propylene glycol (PG) and TC. Nine formulations were subsequently examined in human skin. A good correlation ($r^2 = 0.80$) for methadone permeation was obtained between porcine ear skin and human skin data. Solvent uptake studies indicated that the presence of PG not only increased methadone permeation but also TC permeation. The drug appears to "track" the permeation of TC. Future studies will expand further the range of potential vehicles for optimal delivery of the drug, that will ultimately to be investigated in clinical studies.

Keywords Skin permeation; methadone; propylene glycol; Transcutol® P; synergy;

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Abbreviations:

NMDA	N-methyl-D-aspartate
LIM	d-limonene
EO	Ethyl oleate
TC	Transcutol® P
OSAL	Octyl salicylate
IPA	Isopropyl alcohol
PG	Propylene glycol
HPLC	High performance liquid chromatography
OA	Oleic acid
PGML	Propylene glycol monolaurate
WL 1349	Labrafac™ lipophile WL 1349
PBS	Phosphate buffered saline
DVS	Dynamic vapor sorption
RH	Relative humidity
GC	Gas chromatography
FID	Flame ionization detector
LOD	Limit of detection
LOQ	Limit of quantification
ICH	International Conference of Harmonization
OECD	Organisation for Economic Co-operation and Development
SD	Standard deviation
ANOVA	One-way analysis of variance
TriPG	Tripropylene glycol

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1. Introduction

Methadone is not only a potent synthetic μ-opioid receptors agonist but also a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (McLean and Twomey, 2015). The use of methadone for pain management has received great interest in recent years. The rationale for dermal delivery of methadone has been described previously (Kung et al., 2019). Although topical/transdermal delivery offers advantages over other routes, the formidable barrier properties of the skin limit the percutaneous absorption of many active ingredients and subsequently their therapeutic effects. Our previous studies reported a comprehensive characterization of methadone free base and its hydrochloride salt and suggested that the free base form of methadone has more favourable properties for skin permeation compared with the salt form. A range of vehicles, spanning different physicochemical parameters were also tested. The results for finite dose permeation studies indicated that d-limonene (LIM), ethyl oleate (EO), Transcutol® P (TC) and octyl salicylate (OSAL) were the optimal solvents for skin delivery of the compound (Kung et al., 2019).

The combination of multiple solvents may also have potential for synergistic effects on skin delivery, especially when combining solvents with different mechanisms of penetration enhancement. Binary and ternary systems composed of two or three solvents have been investigated by a number of researchers for dermal delivery of actives (Brinkmann and Müller-Goymann, 2005; Mohammed et al., 2014; Parisi et al., 2016; Haque et al., 2018; Zhang et al., 2019a; Iliopoulos et al., 2020). Recently, Zhang et al. (2019a) reported the permeation of niacinamide from a range of binary and ternary formulations in porcine skin, human skin and an artificial membrane. Enhanced skin delivery was observed for binary and ternary systems compared with single solvents. For example, the inclusion of oleic acid or linolenic acid with propylene glycol (PG) resulted in a ~50-fold increase in the cumulative amount of niacinamide that permeated through human skin after 24 h compared with neat PG. Although co-solvent systems have been explored in this and other studies, the effects of such systems on the skin delivery of methadone have not been reported to date.

Depletion of solvents from the skin surface via either skin permeation or evaporation may alter the thermodynamic activity and, subsequently, permeation of active ingredients. Therefore, monitoring of formulation components should provide insights into how topical products may be better formulated. To our knowledge, PG permeation in human skin was first reported by Hoelgaard and Mollgaard (1985). The authors found a positive relationship between the degree of PG permeation and metronidazole permeation. A “carrier-solvent” effect was therefore proposed by the authors. However, few studies to date have monitored skin

permeation of both active ingredient and vehicle and focus rather on analysing the active ingredients alone. Oliveira et al. (2012) investigated the uptake of isopropyl myristate, dimethyl isosorbide and TC in human skin. The authors suggested that high solvent uptake promoted the stratum corneum partitioning of methyl paraben by modifying the solubility characteristics of the molecule in the skin. More recently, Haque et al. (2017) reported permeation profiles of both anthramycin and four neat solvents, namely PG, 1,3-butanediol (BD), dipropylene glycol and TC. The highest permeation of both solvent and anthramycin through human skin was observed for TC, followed by PG. The authors suggested that anthramycin permeation appeared to "track" the permeation of TC and PG. Subsequently, the same authors reported the effects of binary and ternary systems on the permeation of both anthramycin and solvents (Haque et al., 2018). The inclusion of 10% propylene glycol monocaprylate or propylene glycol monolaurate increased the permeation of both PG and anthramycin compared with using neat PG. This suggests that formulation composition can not only affect the permeation of an active ingredient but also the permeation of the vehicle components themselves. The aims of this study were therefore (i) to investigate binary and ternary vehicles for the dermal delivery of methadone free base in porcine skin (ii) to investigate any synergistic effects on human skin permeation for binary and ternary formulations (iii) to determine the skin uptake and permeation interdependence of both active and selected solvents.

2. Materials and methods

2.1. Materials

Methadone hydrochloride, tripropylene glycol (TriPG), Brij™ O20, OSAL and isopropyl alcohol (IPA) were purchased from Sigma-Aldrich (U.K.). PG, LIM, high performance liquid chromatography (HPLC) grade water, acetonitrile, orthophosphoric acid, 85+%, sodium dihydrogen phosphate and sodium hydrogen phosphate were supplied by Fisher Scientific (U.K.). Oleic acid (OA) and EO were provided by Fluka (U.K.). Labrafac™ lipophile WL 1349 (medium-chain triglycerides of caprylic and capric acids, WL 1349), TC and propylene glycol monolaurate (PGML) Type II were gifts from Gattefossé (St. Priest, France). Phosphate buffered saline (PBS) (pH 7.3 ± 0.2 at 25 °C) was prepared using Dulbecco A tablets supplied by Oxoid (U.K.). Porcine tissue was obtained from a local abattoir on the day of slaughter. Abdominal human skin (Caucasian female donor) was obtained from a tissue bank with institutional ethical approval (Research Ethics Committee reference 07/H1306/98).

2.2. Dynamic vapor sorption (DVS) studies

Dynamic vapor sorption analysis was conducted with a Q5000 SA sorption analyzer (TA Instruments, U.S.A.). The instrument is equipped with an autosampler, a humidity control chamber and a microbalance, allowing accuracy and precision in weight-change detection (accurate to 0.0001 mg). Nitrogen with a flow rate at 200 mL/min was used as the purge gas for the humidity control chamber. The relative humidity (RH) and temperature of the humidity chamber were set to 50 % and 32 °C prior to each experiment. Approximately 5 mg of sample was added into a metal coated quartz pan, placed in the humidity control chamber. The mass change of each sample was monitored over 24 h.

2.3. Gas chromatography (GC) analysis

The GC system used in this study was an Agilent 7890A (G3440A) GC system, equipped with a G4513A autosampler and a flame ionization detector (FID) (Agilent Technologies, U.S.A.). Analysis was performed with a capillary GC column L×I.D. 30 m×0.32 mm with a 0.25 µm stationary phase HP 5® [(5%-Phenyl)-methyl polysiloxane] (Agilent J & W GC column, U.S.A.). Data were collected and analyzed with OpenLAB CDS ChemStation ® Edition Rev C.01.04 (Agilent Technologies, U.S.A.). Nitrogen was used as the carrier gas at a flow rate of 2 mL/min. The inlet temperature and purge flow were set to 250 °C and 40 mL/min at 0.75 min, respectively. 5 µL of sample was injected in the splitless mode. The detector temperature was set at 325 °C. The initial oven temperature was set at 50 °C, ramped to 80 °C at 10 °C/min, held at 80 °C for 2 min, then ramped to 100 °C at 10 °C/min, held at 100 °C for 4 min, and finally ramped to 300 °C at 80 °C/min and held at 300 °C for 8 min. The GC method was validated in terms of specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to the International Conference of Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005). Calibration curves for PG and TC within the concentration range of 0.04 – 1 µL/mL were constructed ($r^2 \geq 0.99$) and the LOQ values for PG and TC were 0.09 and 0.08 µL/mL, respectively.

2.4. HPLC analysis

The HPLC method for the analysis of methadone has been reported previously by Kung et al. (2019). The method was validated following ICH guidelines Q2 (R1) (ICH, 2005). Calibration curves for methadone within the concentration range of 0.5–100 µg/mL were constructed ($r^2 \geq 0.99$) with the LOQ of 0.69 µg/mL.

2.5. *In vitro* finite dose permeation and mass balance studies

Full-thickness porcine ear skin was separated from the cartilage while the excised epidermis of human skin was prepared by the heat separation method (Kligman and Christophers, 1963). The skin was stored at −20 °C until required. *In vitro* permeation studies were conducted according to the Organisation for Economic Co-operation and Development (OECD) guideline No. 428 (OECD, 2004a) using custom-made vertical glass Franz diffusion cells (Soham Scientific, U.K.). Details have also been reported previously by Santos et al. (2010). The diffusion area was ~1 cm^2 and was accurately measured using a Vernier Callipers (Fisher Scientific, U.K.). PBS pH 7.3 ± 0.2 with the addition of 6% w/v Brij™ O20 was used as the receptor medium to ensure sink conditions during permeation studies (Bronaugh and Stewart, 1984; Brain et al., 2002). The receptor compartment was filled with ~ 2.1 mL of the receptor medium. Finite doses (5 μL/cm^2) of 5% w/v methadone base formulations were applied. Permeation studies were conducted over 24 h at 32 ± 1 °C. The receptor medium was sampled at 0, 2, 4, 6, 8, 10, 12, 24 h after the formulation application. Mass balance studies were validated and conducted after each permeation study as reported previously as described by Kung et al. (2019). Samples from permeation and mass balance studies were analyzed by HPLC and GC.

2.6. Statistical analysis

Data processing and the calculation of mean and standard deviation (SD) were determined using Microsoft Excel® 2016 (Microsoft, U.S.A.). Statistical analysis was performed using IBM® SPSS Statistics® 24.0 (IBM SPSS Statistics, U.K.). The normality of the data was assessed using the Shapiro-Wilk test. For the parametric data that were assumed to be normally distributed, the independent-sample t test and one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test were used to analyze two groups and ≥3 groups respectively. For the non-parametric data (non-normally distributed data), the Mann-Whitney U test was used to test statistical significance between two groups and the Kruskal-Wallis one-way ANOVA test was performed to investigate statistical differences between different groups (≥ 3 groups). A *p*-value lower than 0.05 was considered as a statistically significant difference.

3. Results and discussion

3.1. Formulation evaporation studies using DVS

If a solvent component of a drug solution evaporates after application, it will potentially increase the concentration of the active on the skin surface. Eight solvents that we have

investigated in our previous skin permeation studies were selected to evaluate their volatility. The results for formulation evaporation studies under static conditions (32 ± 1 °C and 50 ± 1.5% RH) are shown in Figure 1. The weight of TC and TriPG increased during the first 15 min because of the hygroscopic nature of these solvents. LIM evaporated completely by 45 min with approximately 5% of the applied mass remaining, namely the residual methadone free base, in the sample pan. The steady-state evaporation rates of LIM and TC were 84.37 ± 1.13 and 5.10 ± 0.42 µg/min, respectively. Less than 2 % of the applied PGML, TriPG, OSAL, EO, OA and WL1349 formulations evaporated over 24 h. The results for TC, PGML and OSAL were consistent with previous studies (Haque et al., 2017; Paz-Alvarez et al., 2018).

3.2. *In vitro* finite dose permeation and mass balance studies

3.2.1. Binary systems in porcine skin

In our previous methadone permeation studies on single solvent systems, LIM, EO, TC and OSAL appeared to be promising candidate components of dermal formulations for methadone delivery. PG is extensively used in dermal formulations and the Cosmetic Ingredient Review Expert Panel has concluded that PG is safe as used in cosmetic formulations (Fiume et al., 2012). A number of studies have reported the use of PG for skin delivery of actives (Barrett et al., 1965; Poulsen et al., 1968; Coldman et al., 1969; Davis et al., 1981; Hoelgaard and Mollgaard, 1985; Kasting et al., 1993; Arellano et al., 1999; Trottet et al., 2004; Nicolazzo et al., 2005; Watkinson et al., 2009; Zhang et al., 2011). However, neat PG was not taken forward for investigation because of the low solubility of methadone free base in PG (24.78± 0.49 mg/mL at 32 ± 1 °C). TC was used to increase the solubility of methadone in formulations containing PG (solubility data are shown in Figure S1). A binary PG:TC system was therefore selected in order to investigate the effects of PG and TC on methadone permeation.

The cumulative amounts of methadone that permeated through porcine skin from binary systems are shown in Figure 2 and the mass balance results are summarized in Table S1. Previous studies with neat solvents confirmed that LIM delivered the highest amount of methadone through porcine skin (Kung et al., 2019). The results from the present work show that, with the exception of the EO/OSAL formulation, the binary formulations delivered significantly higher ($p < 0.05$) amounts of methadone through porcine skin compared with LIM. The percentages of applied methadone remaining on the skin surface ranged from 15.33% to 46.03%. In addition, the highest permeation of methadone observed was 161.01 ± 19.66 µg/cm^2 (73.74 ± 8.15% of applied dose) for the PG:TC (50:50) formulation after 24 h, significantly

higher than other formulations presented in Figure 2 ($p < 0.01$). The PG:TC (50:50) formulation promoted a 40-fold increase in the cumulative amount of methadone that permeated through porcine skin at 12 h compared with neat TC.

To investigate further the roles of PG and TC for formulation optimization, various ratios of PG:TC from 25:75 to 75:25 were selected for permeation and mass balance studies (data are shown in Figure. 3 and Table S1). Both PG:TC (25:75) and PG:TC (50:50) appear to be promising vehicles for methadone free base and no significant difference ($p > 0.05$) was observed between the permeation values. PG:TC (25:75) delivered 152.07 ± 26.20 μg/cm^2 of methadone through porcine skin after 24 h. The pseudo steady state skin fluxes for PG:TC (25:75) and PG:TC (50:50) were 10.01 ± 2.81 and 13.11 ± 2.60 μg/cm^2/h, respectively. These values were significantly higher ($p < 0.01$) than the maximum value (2.54 ± 0.19 μg/cm^2/h) obtained for the single solvent systems. However, the percentage of applied methadone that permeated through skin for PG:TC (75:25) was significantly lower compared with other PG/TC binary formulations ($p < 0.01$). One reason for this result is that drug crystallization occurred. Methadone crystals were found on the skin surface during permeation (Figure S1). When the depletion of solvents from skin surface (via skin uptake or evaporation) is far greater than that for methadone, drug crystallization may occur, notably for PG:TC (75:25) where the solubility of methadone is relatively low (Figure S2). Results from mass balance studies are consistent with these findings (Table S1). 18% and 15% of the applied dose of methadone remained on the skin surface for PG:TC (25:75) and PG:TC (50:50), respectively, while 48% of the applied dose of methadone from PG:TC (75:25) remained on the skin surface. The total recovery values for all binary formulations were within the recovery range (90% – 110%) recommended by the relevant OECD guidelines (OECD, 2004b).

3.2.2. Ternary systems in porcine skin

Ternary systems composed of PG, TC and IPA or LIM were also evaluated and the permeation profiles are shown in Figure 4. The cumulative amounts of methadone that permeated through porcine skin for PG:TC:IPA (40:40:20) and PG:TC:IPA (30:30:40) were significantly higher ($p < 0.01$) than for the single solvent systems. The pseudo steady state skin fluxes for PG:TC:IPA (40:40:20) and PG:TC:IPA (30:30:40) were 11.98 ± 2.89 and 8.23 ± 0.90 μg/cm^2/h, respectively. Results of the mass balance studies for ternary formulations are shown in Table S1. The percentages of methadone recovered from skin extraction for PG/TC/IPA ternary formulations were significantly higher ($p < 0.05$) compared with PG:TC (50:50). The concentrations of TC in PG/TC/IPA ternary formulations were relatively lower

compared with the PG:TC (50:50) formulation. Methadone permeation may be influenced by the depletion of TC in the skin. This might explain why the PG/TC/IPA ternary formulations delivered low ($p < 0.05$) amounts of methadone through the skin compared with PG:TC (50:50).

Although LIM is generally recognized as a safe ingredient in topical products (Herman and Herman, 2015), the highest concentration that has been used in FDA approved topical products is 10% w/w (FDA, 2018). Therefore, only 5% w/v of LIM was incorporated in the PG/TC/LIM ternary formulations. There was no significant difference ($p > 0.05$) in the cumulative amounts of permeation of methadone for PG:TC (50:50), PG:TC:LIM (45:50:5) and PG:TC:LIM (65:30:5). In contrast, the permeation of methadone for the PG:TC:LIM (25:70:5) formulations was significantly lower ($p < 0.05$) compared with the PG/TC/LIM ternary formulations containing higher concentrations of PG. This suggests that the dose of PG may play an important role in enhancing permeation of methadone as reported for loperamide by Trottet et al. (2004). Although the PG dose-dependent effect for PG/TC binary systems was not observed in porcine skin, this effect was observed in human skin (see Figure 5 below in Section 3.2.3). Methadone permeation from PG:TC (50:50) 24h post application in human skin was significantly higher ($p < 0.01$) compared with PG:TC (25:75). One possible reason why no statistically significant difference between PG:TC (25:75) and PG:TC (50:50) was observed in porcine skin is that the effect may be "masked" by the variability of porcine skin permeability.

3.2.3. Single, binary and ternary systems in human skin

To confirm the synergistic action of binary and ternary systems, some of the formulations that were tested in porcine skin were also evaluated in human skin. The results of the permeation and mass balance studies are shown in Figure. 5 and Table S2, respectively. A synergistic action of PG and TC was observed and PG:TC (50:50) delivered the highest amount ($p < 0.05$) of methadone through skin after 24 h compared with other formulations tested in human skin. Unlike permeation studies in porcine skin, the finite dose permeation profiles in human skin did not show a classic plateau for most formulations, with the exception of PG/TC/IPA systems. It is notable that the PG:TC:IPA system delivered significantly higher ($p < 0.05$) amounts of methadone through human skin than the PG:TC (50:50) formulation up to 12 h. Compared with TC, an 8.9-fold increase in the cumulative amount of methadone that permeated at 12 h was observed for PG:TC (50:50) while a 21.5-fold increase was observed for PG:TC:IPA (40:40:20). After 12 h, a plateau for methadone permeation was observed for PG:TC:IPA (40:40:20) and PG:TC:IPA (30:30:40) that may reflect the depletion of methadone.

Rapid clearance of vehicles may also lead to this plateau when vehicle permeation is far faster than methadone, which would result in methadone crystallization in the skin. Further permeation of methadone was therefore limited. This might explain why relatively high deposition of methadone in the skin was observed for PG:TC:IPA (30:30:40) in mass balance studies (Table S2). For an analgesic, predictable rates of absorption and time to known steady state plasma or tissue levels will be required for efficacy in clinical use. The lag times of methadone permeation for PG:TC (50:50) and PG:TC:IPA (40:40:20) were 6.55 ± 0.32 h and 3.59 ± 0.21 h, respectively. This indicates that the inclusion of a volatile solvent in PG/TC binary formulations may be a favourable strategy for dermal delivery of methadone. Patients may benefit from the shorter lag time and relatively high amounts of methadone permeation within 12 h post application.

3.3. Correlation of methadone permeation in porcine skin and human skin

The relationship between methadone permeation in porcine skin and human skin is shown in Figure 6. The results confirm that, for methadone, porcine skin is 2-5 times more permeable than human skin, which is consistent with previous studies (Dick and Scott, 1992; Singh et al., 2002; Boudry et al., 2008; Vallet et al., 2008; Zhang et al., 2019b). A positive correlation between the cumulative amount of methadone permeation in porcine ear skin and human skin was observed ($r^2 = 0.80$) in this study. This confirms the utility of porcine ear skin for preliminary studies to optimize methadone formulations.

3.4. Investigation of the synergistic action of PG and TC – skin permeation of PG and TC

The human skin absorption and permeation of TC and PG were reported recently by our group (Haque et al., 2017; Haque et al., 2018). In the present work the percentages of applied TC and PG that permeated through porcine skin and human skin are shown in Figure 7. The percentage of PG that permeated through porcine ear skin after 24 h was significantly higher ($p < 0.01$) compared with permeation through human skin. More than 50% of the applied PG permeated from PG:TC binary systems through porcine skin while less than 15% of applied PG permeated through human skin. Rapid uptake of TC was observed for both porcine skin and human skin. 65-70% of TC permeated through porcine skin from the PG:TC binary formulations after 24 h while only 31% of TC permeated from the neat TC solution. This indicates that the presence of PG in formulations may facilitate the permeation of TC; a similar trend was also observed in human skin. At 24 h, the values for percentage permeation of TC through human skin for PG:TC (25:75) and PG:TC (50:50) were 32% and 51% respectively

while the corresponding value for neat TC was only 13%.

The synergistic action of PG with other vehicles on skin permeation has previously been observed (Haque et al., 2018; Zhang et al., 2019a; Iliopoulos et al., 2020). These finite dose studies reported that permeation enhancement was achieved by combining PG with lipophilic solvents such as isopropyl myristate, oleic acid, linolenic acid and propylene glycol monolaurate. The mechanism for the synergistic actions of PG with TC remains to be elucidated. Although synergistic actions between PG and TC on penetration enhancement have not been detailed in research articles, two patents assigned to Antares Pharma include pertinent data. US8980309 B2 disclosed the use of PG and TC at different ratios to inhibit testosterone crystallization in gel formulations (Carrara and Grenier, 2015). *In vitro* porcine skin permeation studies were also reported in the patent. The gels containing 5% w/w TC and 6-30% w/w PG led to increases in the skin permeation and skin extraction of testosterone compared with the control formulation after 24 h permeation studies. However, the mechanisms of synergistic actions from the combined use of PG and TC were not reported in the patent. US20150005337 A1 reported that hydroalcoholic solutions containing PG and TC delivered increased amounts of various central nervous system acting drugs such as rivastigmine and fentanyl through porcine skin compared with controls (Carrara et al., 2015). The control hydroalcoholic solutions consisted primarily of an active ingredient, ethanol, water, pH adjusters and hydroxypropyl cellulose. *In vitro* permeation studies described in this patent have confirmed synergistic effects from the PG and TC with fatty alcohols such as myristyl alcohol and lauryl alcohol. The results from the present work are consistent with these patent disclosures.

The mechanisms of penetration enhancement of PG have been reviewed by Lane (2013). The incorporation of PG into the polar regions of the lipid bilayers may modify the physical and chemical properties of the intercellular lipids and thus enhance the skin permeation of TC and methadone. Bouwstra et al. (1991) compared the arrangement of the lipid bilayers in PG pre-treated and untreated human stratum corneum using small-angle X-ray scattering. The authors suggested that PG may not lead to the swelling of lipid bilayers because there was no change in the repeat distances between the lipid lamellae for PG treated skin. The authors also concluded that PG may be incorporated into the head group regions without changing the repeat distances between the lipid lamellae rather than intercalating in the lipid bilayers. Brinkmann and Müller-Goymann (2005) also suggested that PG may increase the mobility of actives and excipients in the lipid lamellae.

The presence of PG increased not only the permeation of methadone but also the permeation of TC. The increased skin uptake of TC may increase further the permeation of

methadone by changing the skin environment for percutaneous absorption. Figure 8 compares the permeation profiles of methadone, PG and TC in human skin. A good correlation (r^2 = 0.97) between the amount of methadone and TC permeated at 24 h was observed. The results suggest that the permeation of methadone followed the permeation of TC closely.

A further potential mechanism of the penetration enhancement of PG on basic drugs is also hypothesised. Skin lipids are mainly composed of various types of ceramides, free fatty acids, cholesterol and cholesteryl sulphate (Marjukka Suhonen et al., 1999; Walters and Roberts, 2002). The free fatty acids in the epidermis may interact with basic drugs such as methadone, rivastigmine and fentanyl. Therefore, the free base forms of these drugs may be converted to the less hydrophobic protonated forms which are generally less permeable compared with free base forms. Reports in the literature suggest that PG can react with free fatty acids under specific *in vitro* conditions (Shaw and Lo, 1994; Shaw et al., 2003). Therefore, it is possible that the presence of PG may enhance the permeation of methadone by interacting with the carboxyl group of fatty acids in skin. Recent studies have also revealed the important role of skin microbiota in skin lipid composition and barrier function (Myles et al., 2016; Chen et al., 2018; Niehues et al., 2018). Further investigations of potential skin microbiota involvement in the interactions between the carboxyl group of free fatty acids and the hydroxyl group of PG in skin are necessary in order to clarify this possibility.

4. Conclusions

The effects of binary and ternary systems on the dermal delivery of methadone were investigated in this proof-of-concept study. Enhanced skin permeation was observed for all formulations tested, compared with previous work, especially for formulations incorporating both PG and TC. The maximum skin flux obtained for permeation studies in porcine skin and human skin was 13.58 ± 4.16 and 3.57 ± 0.48 μg/cm^2/h, respectively. Although methadone free base has poor solubility in PG, the presence of PG in binary systems with TC appears to play an important role in the delivery of the molecule. The mechanisms for the synergistic action of PG and TC were explored by monitoring the permeation of both solvents. TC was proposed as a good "carrier-solvent" for methadone and results from the present work confirm that the permeation of methadone appears to "track" the permeation of TC. Solvent uptake studies show that the presence of PG in binary formulations increased not only the permeation of methadone but also the skin uptake of TC. Further investigation of the biochemical reactions between PG and free fatty acids in the skin is required to understand any potential mechanisms of PG *in vivo*. This study should contribute to the development of novel dermal formulations for

methadone and will ultimately provide alternative approaches for management of pain. Other techniques to study the synergistic actions of PG and TC at the molecular level, for example, confocal Raman spectroscopy, should also allow a better understanding of their residence time and effects on the skin.

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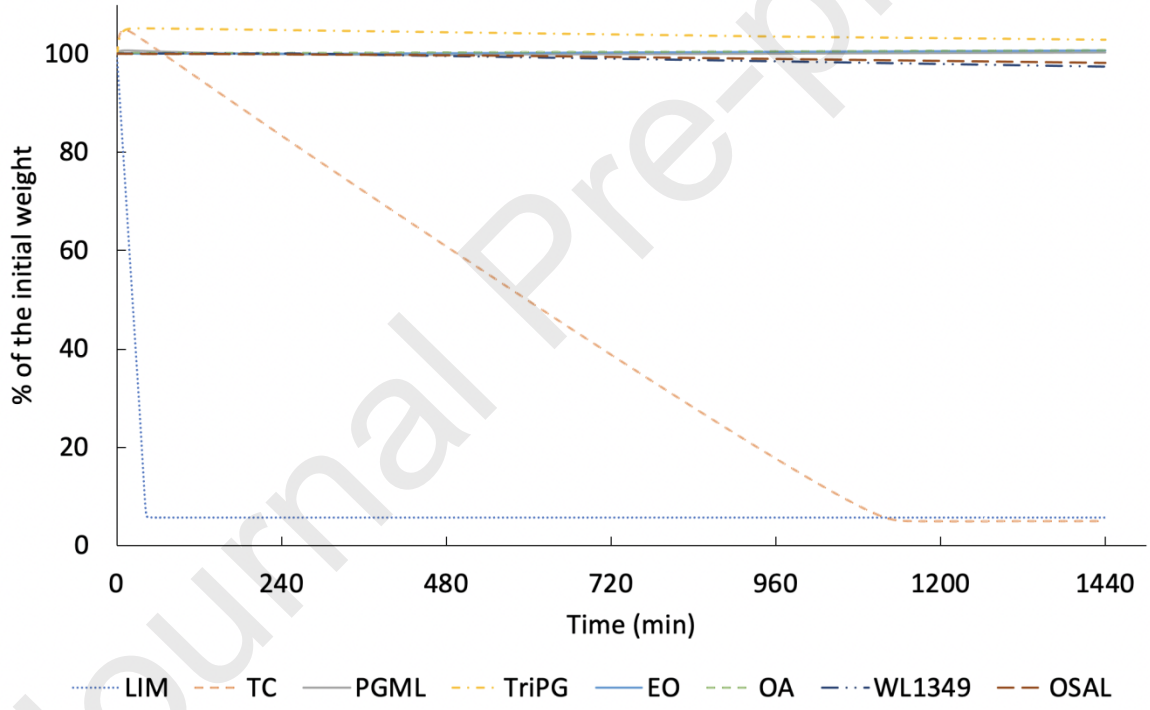

Figure 1. Percent of the applied weight of 5% (w/v) methadone free base solutions remaining over 24 h under static condition (32 ± 1 °C and 50 ± 1.5% RH) (mean ± SD; n=3).

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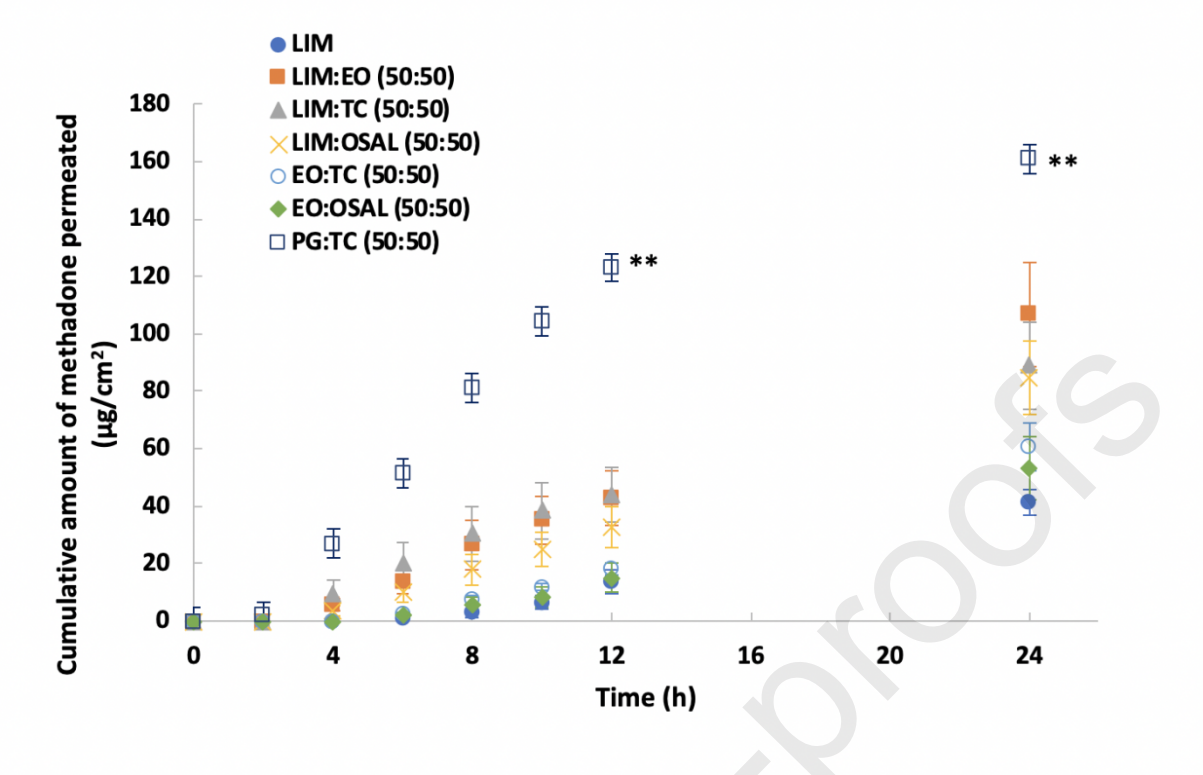

Figure 2. Permeation profiles of methadone in porcine ear skin after application of 5% (w/v) methadone free base in LIM (●), LIM:EO (50:50) (■), LIM:TC (50:50) (▲), LIM:OSAL (50:50) (×), EO:TC (50:50) (○), EO:OSAL (50:50) (◆) and PG:TC (50:50) (□) for finite dose (5 µL/cm^2) at 32 ± 1 °C (mean ± SD; $4 \leq n \leq 5$).

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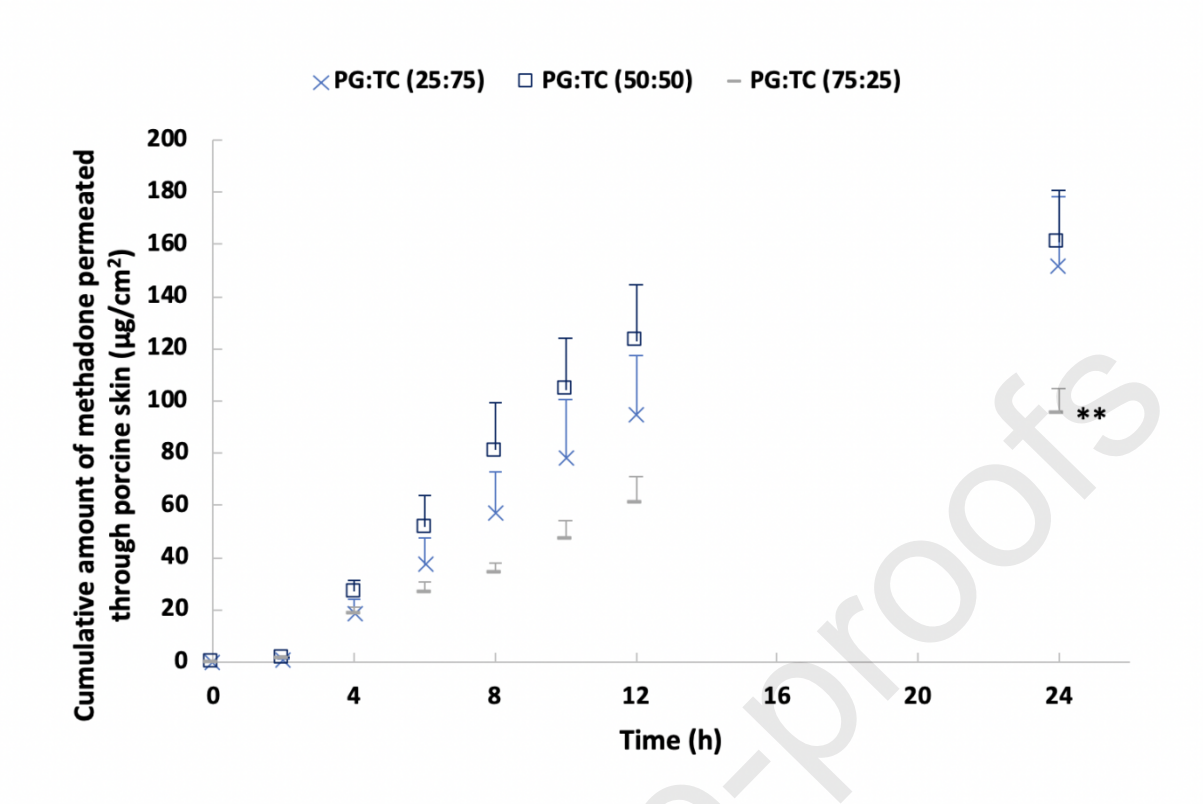

Figure 3. Permeation profiles of methadone in porcine ear skin after application of 5% (w/v) methadone free base in PG:TC (25:75) (×), PG:TC (50:50) (□) and PG:TC (75:25) (–) for finite dose (5 µL/cm^2) at 32 ± 1 °C (mean ± SD; 4 ≤ n ≤ 5).

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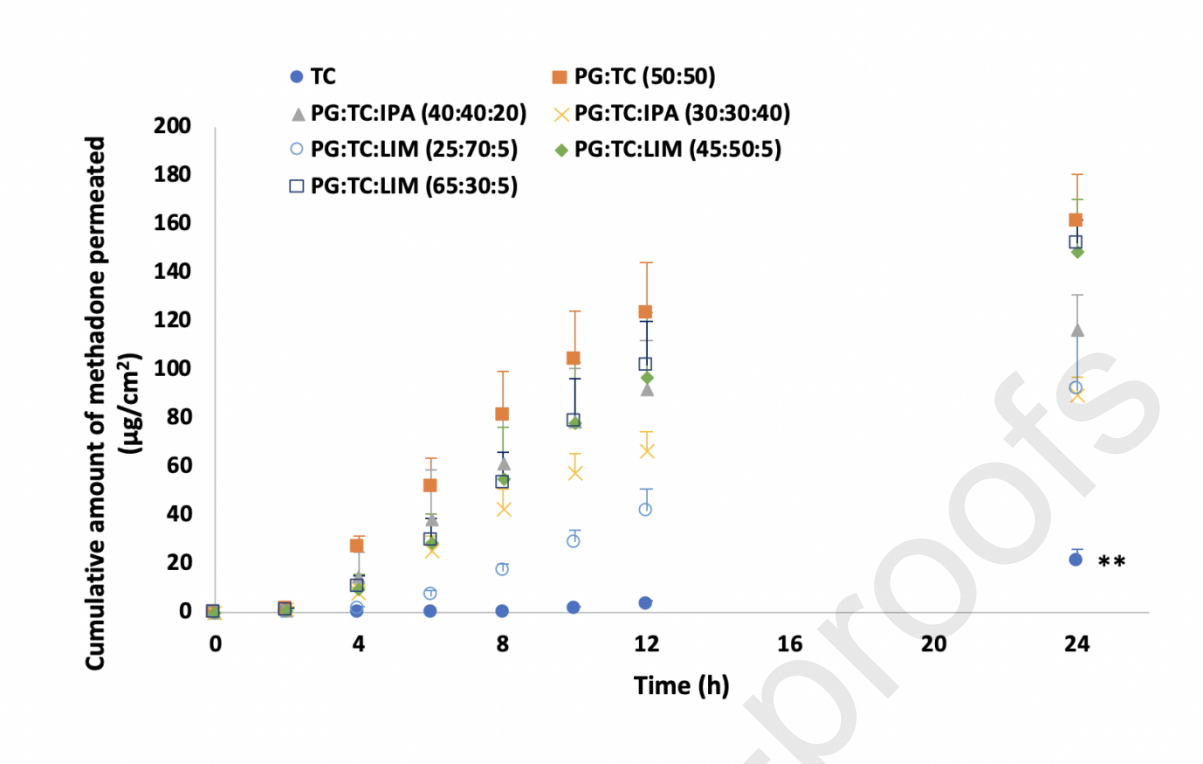

Figure 4. Permeation profiles of methadone in porcine ear skin after application of 5% (w/v) methadone free base in TC (●), PG:TC (50:50) (■), PG:TC:IPA (40:40:20) (▲), PG:TC:IPA (30:30:40) (×), PG:TC:LIM (25:70:5) (○), PG:TC:LIM (45:50:5) (◆) and PG:TC:LIM (65:30:5) (□) for finite dose (5 µL/cm^2) at 32 ± 1 °C (mean ± SD; 4 ≤ n ≤ 5).

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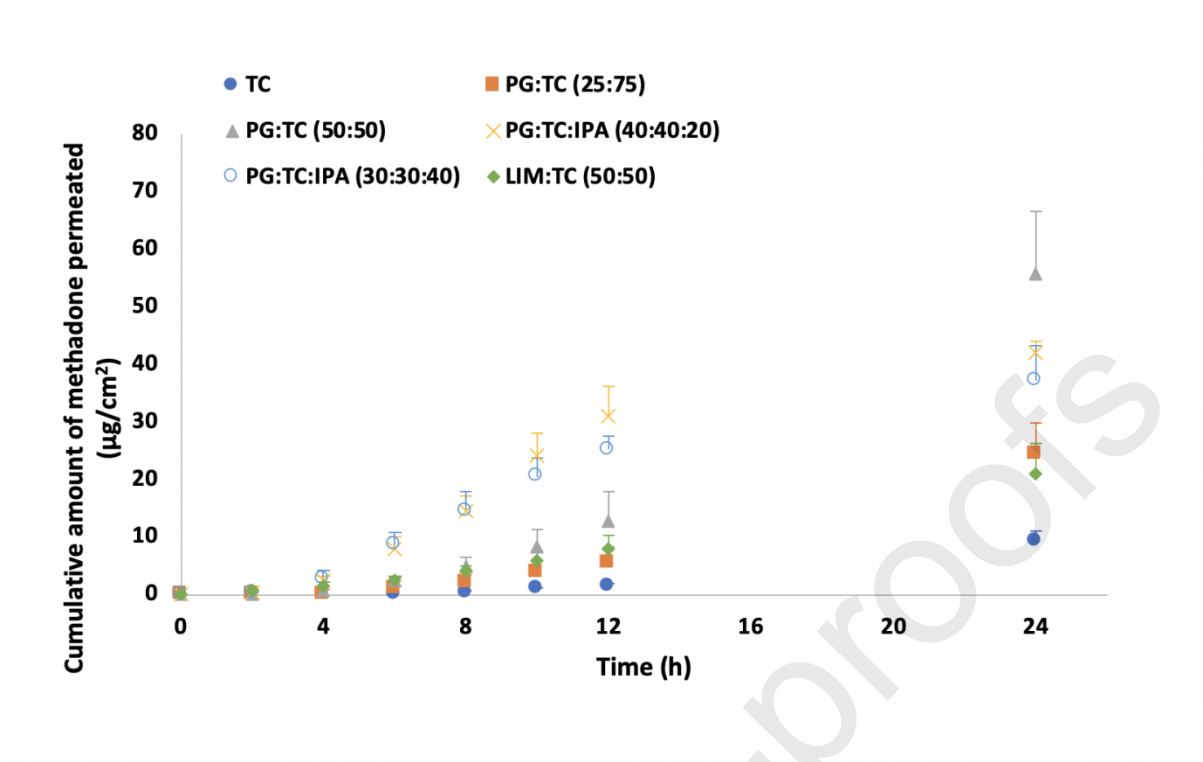

Figure 5. Permeation profiles of methadone in human skin after application of 5% (w/v) methadone free base in TC (●), PG:TC (25:75) (■), PG:TC (50:50) (▲), PG:TC:IPA (40:40:20) (×), PG:TC:IPA (30:30:40) (○) and LIM:TC (50:50) (◆) for finite dose (5 µL/cm^2) at 32 ± 1 °C (mean ± SD; 4 ≤ n ≤ 5).

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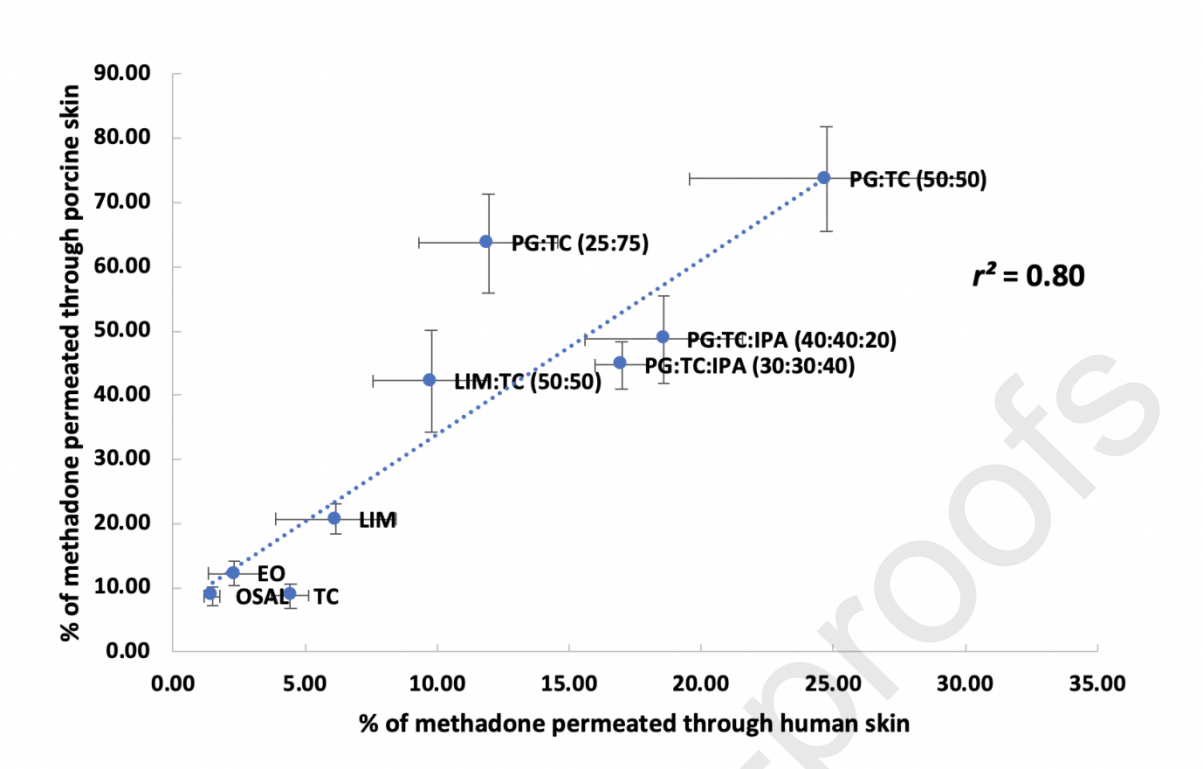

Figure 6. Correlation between the percentage of applied methadone permeated through human skin and porcine skin after 24 h (mean ± SD; $4 \leq n \leq 5$).

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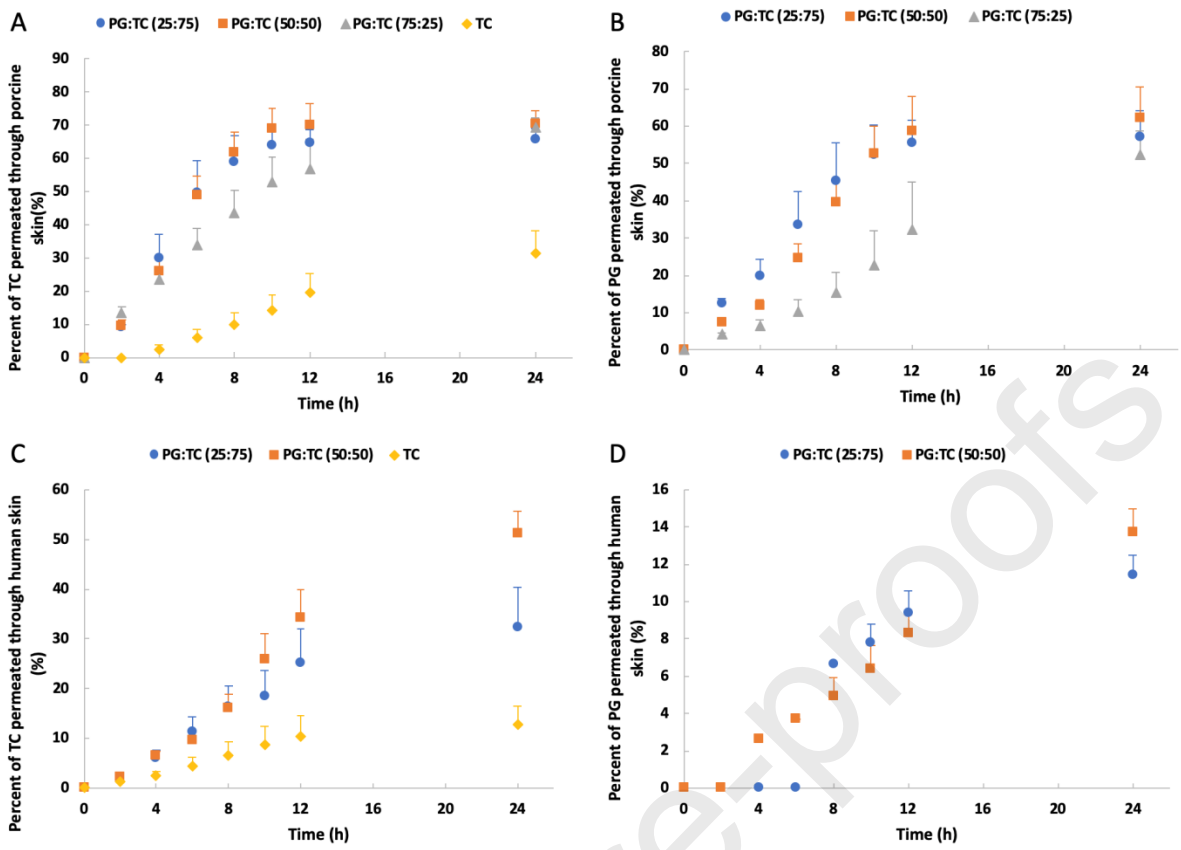

Figure 7. Permeation profiles for solvent uptake in porcine skin and human skin after application of 5% (w/v) methadone free base in PG:TC (25:75) (●), PG:TC (50:50) (■), PG:TC (75:25) (▲), TC (◆) finite doses (5 µL/cm^2) at 32 ± 1 °C (mean ± SD; n = 4).

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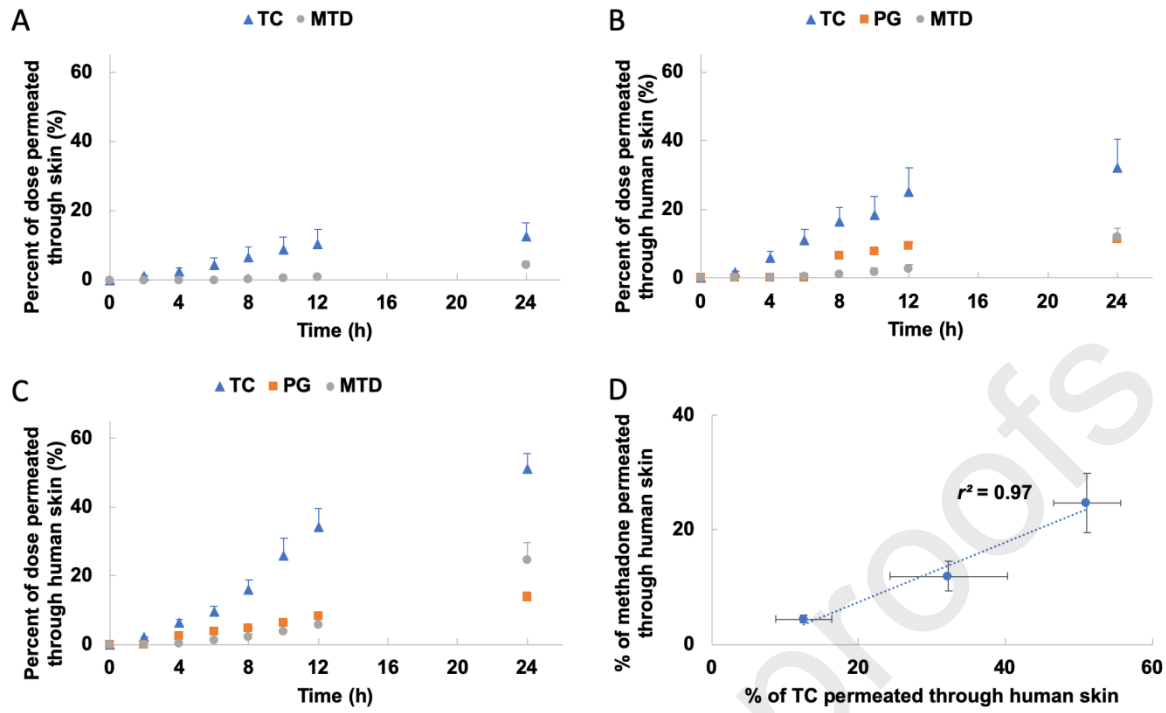

Figure 8. Comparison of permeation profiles of methadone (●), PG (■) and TC (▲) in human skin under finite dose conditions (5 µL/cm²) at 32 ± 1 °C (mean ± SD; n = 4). The 5% w/v methadone base formulations applied were prepared in **(A)** neat TC, **(B)** PG:TC (25:75) and **(C)** PG:TC (50:50). **(D)** Relationship between the percentages of methadone and TC permeated at 24 h.

Chin-Ping Kung: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Yanling Zhang:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing - review & editing. **Bruno C. Sil:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing - review & editing. **Jonathan Hadgraft:** Conceptualization, Supervision, Visualization, Writing - review & editing. **Majella E. Lane**: Conceptualization, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Bhumik Patel:** Writing - review & editing. **Renée McCulloch:** Conceptualization, Writing - review & editing

Declaration of interests

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☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- A good correlation ($r^2 = 0.80$) for methadone permeation was obtained between porcine ear skin and human skin
- Propylene glycol increased the skin permeation of Transcutol® in porcine skin and human skin
- Synergistic actions between propylene glycol and Transcutol® on the penetration enhancement of methadone were observed
- Methadone appears to "track" the permeation of Transcutol®

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