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Ion pairing as a strategy to enhance the delivery of diclofenac

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This study explores the use of ion pairing and solvent selection to enhance the percutaneous delivery of diclofenac (DF) from topical formulations. Previous investigations identified L-histidine monochloride monohydrate (LHSS) as an ion pair candidate for diclofenac sodium (DNa). Initial *in vitro* permeation tests (IVPT) demonstrated that while LHSS increased DF permeation, it caused DF precipitation at higher concentrations. As DNa is sparingly soluble in water, the only solvent in which LHSS dissolves, its solubility was tested in alternative solvents. The highest solubility was observed in Transcutol® (TC), dipropylene glycol (DiPG) and propylene glycol (PG). Building on earlier research using TC : water binary systems to evaluate ion pairs, this study assessed: (i) the substitution of TC with DiPG in binary formulations, (ii) the development of ternary systems comprising water, TC and either DiPG or PG, and (iii) their impact on DF delivery using finite dose IVPT with porcine skin. The inclusion of LHSS (10 mg mL^{-1}) with DNa (10 mg mL^{-1}) in a DiPG : water (60 : 40 v/v) binary system significantly enhanced DF delivery (2.69 ± 1.01%), relative to the LHSS-free control (1.02 ± 0.44%, $p < 0.05$.). However, this was significantly lower than in TC : water binary formulations (4.80 ± 1.08–5.41 ± 2.21%; $p < 0.05$). Similarly, the ternary formulation containing DiPG (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50 v/v/v) resulted in lower DF delivery (5.62 ± 2.78%) compared to the corresponding TC : water (50 : 50 v/v) binary formulation (12.26 ± 3.06%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS, $p < 0.05$). Conversely, replacing DiPG with PG in the ternary formulation (PG : TC : water; 10 : 40 : 50 v/v/v) containing 25 mg mL^{-1} LHSS, significantly enhanced DF permeation (4.26 ± 1.41 μg cm^{-2}) compared to all binary (0.14 ± 0.28–1.52 ± 0.32 μg cm^{-2}) and ternary formulations (0.21 ± 0.36–1.72 ± 1.06 μg cm^{-2}, $p < 0.05$). This formulation also outperformed a recognised commercial product (1.74 ± 0.6 μg cm^{-2}) by 145%, despite containing only half the DNa concentration and resulted in the highest total DF uptake as a percentage of the applied dose (27.25 ± 2.61%). This work builds on previous findings, confirming that LHSS enhances DF delivery in combination with DNa. By examining solvent systems and counterion effects, it provides a deeper understanding of formulation strategies to optimise the percutaneous delivery of DF.

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Introduction

Osteoarthritis (OA) affects over 500 million people worldwide, with substantial direct and indirect economic costs.[1,2] Topical non-steroidal anti-inflammatory drugs (NSAIDs), and diclofenac (DF) in particular, are widely recommended for the treatment of OA due to their effectiveness and lower risk of adverse effects compared to oral NSAIDs and opioids.[3–7] However, due to the barrier function of the skin's stratum corneum (SC), only a small fraction of the applied drug penetrates effectively, leaving much of it unable to reach the target site. Improved formulation of DF products could lead to both cost savings and reduced environmental impact by minimising excess drug waste. This aligns with the environmental initiatives of major pharmaceutical companies, like Astra Zeneca,[8] Novartis,[9] and Roche,[10,11] which aim to reduce pharmaceutical residues in the environment where possible.

Previous research by the authors investigated ion pairing to enhance the percutaneous delivery of DF.[12,13] Initial distribution coefficient studies showed that adding L-histidine monochloride monohydrate (LHSS) to aqueous diclofenac sodium (DNa) solutions increased the amount of DF partitioning from the aqueous to the organic phase, with higher LHSS amounts leading to greater DF transfer to the organic layer. Subsequent infinite dose *in vitro* permeation tests (IVPT) using porcine skin indicated that incorporating LHSS in DNa formulations enhanced DF permeation compared to formulations without LHSS. The aqueous formulations investigated also highlighted solubility challenges. As DNa is only sparingly

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soluble in water,[14] the sole solvent in which LHSS dissolves, its solubility was tested in various alternative solvents. Of these, Transcutol® (TC), propylene glycol (PG) and dipropylene glycol (DiPG) were identified as the solvents in which DNa was most soluble.[13]

The authors identified TC : water as a model binary solvent system for the evaluation of the DNa : LHSS ion pairs, as it enabled the comparison of a variety of formulations. This selection of TC as a solvent resulted in a large increase in the solubility of DNa,[13] addressing a major challenge identified in earlier experimental work.[12] However, when TC exceeded a 50 : 50 (v/v) ratio in TC : water, it appeared to reduce the thermodynamic activity of DNa in the formulation. This in turn led to a significant reduction in the movement of DF into and through the membrane, indicating the importance of optimising for the competing aspects of solubility and thermodynamic activity when choosing the solvents for a formulation. To further investigate the effects of solvents on the DNa–LHSS ion pair, this study has three objectives: (i) to replace TC with an alternative solvent in a binary formulation; (ii) to develop ternary systems incorporating water and solvents in which DNa has previously shown high solubility; and (iii) to conduct IVPT, applying finite doses (10 μL) to porcine skin to evaluate the impact of these formulations on the percutaneous delivery of DF.

Materials and methods

Materials

Diclofenac sodium (DNa) 98%, L-histidine monochloride monohydrate (LHSS) and dipropylene glycol (DiPG) were produced by Acros Organics and supplied by VWR (Leceistershire, UK). Voltaren® 1% gel (Haleon, New Jersey, USA) was purchased from Walgreens (New York, USA). High vacuum grease was obtained from Dow Corning (Seneffe, Belgium). Oxoid™ Phosphate buffered saline (PBS) tablets were purchased from Thermo Fisher Scientific (Lancashire, UK). HPLC grade acetonitrile (ACN), trifluoroacetic acid (TFA), methanol and 150 mm diameter filter paper, were purchased from Fisher Scientific (Lancashire, UK). Propylene glycol (PG) was produced by Sigma-Aldrich and supplied by Merck Life Sciences (Poole, UK). Diethylene glycol monoethyl ether, with the trade name Transcutol® (TC), was kindly donated by Gattefossé (St Priest, France). Ion pairs were generated *in situ*, with formation confirmed by FT-IR spectroscopy (SI).

HPLC analysis

Detection and quantification of DF was carried out using the method previously reported by the authors.[12,13] Validation for linearity, accuracy, precision and robustness, as well as limits of detection and quantification was performed in accordance with the International Conference on Harmonisation Expert Working Group (ICH) guidelines (2005).[15] The mobile phase comprised acetonitrile and 0.1% trifluoroacetic acid in water, in a ratio of 70 : 30 (v/v). The injection volume was 10 μL and the flow rate was 1 mL min^{-1}. A detection wavelength of 277 nm was selected for the acquisition of chromatograms. Calibration curves ranging from 0.05–100 μg mL^{-1} DF, were prepared using DNa. The limit of detection was 0.03 μg mL^{-1}, while the limit of quantification was 0.10 μg mL^{-1}.

Binary solvent systems

To determine the effects of a non-aqueous solvent substitution on the movement of DF into and through the membrane, TC was replaced with DiPG. Studies previously identified DiPG as one of three solvents in which DNa exhibited the highest solubility relative to the other solvents tested.[13] The original binary solvent formulations comprising TC and water (60 : 40 v/v), and containing 10 mg mL^{-1} DNa, and either 0 or 10 mg mL^{-1} LHSS[13] were selected for TC substitution. In the modified formulations, TC was replaced with DiPG while maintaining the 60 : 40 (v/v) solvent ratio with water (60 : 40 v/v).[13]

Miscibility testing of drug-loaded ternary solvent systems

Ternary solvent combinations comprised an aqueous component ranging from 50–80% (v/v). The minimum water requirement was previously established to ensure a sufficient quantity of LHSS in the preparation, as well as to enhance the thermodynamic activity of DNa in the formulation.[13] Where the aqueous fraction amounted to 50% (v/v), the remaining 50% (v/v) was apportioned between the non-aqueous solvents in ratios of 40 : 10, 30 : 20, 20 : 30 and 10 : 40 (v/v). When the aqueous volume represented 60% (v/v), the 40% (v/v) attributable to non-aqueous solvents was divided on a 30 : 10, 20 : 20 and 10 : 30 (v/v) basis. When the aqueous fraction was 70% (v/v), the non-aqueous fraction comprised the combinations 20 : 10 and 10 : 20 (v/v). Finally, when the aqueous proportion was 80% (v/v), the non-aqueous solvents each represented 10% (v/v). Methylene blue was added to the solvent combinations to confirm miscibility.

The studies were performed using DNa in fixed concentrations of 1.00%, 0.75% and 0.50% (w/v). Stock solutions of LHSS in water were prepared at concentrations of 50 mg mL^{-1} and 25 mg mL^{-1}. Like the previously conducted binary studies,[13] the concentration of DF remained constant, regardless of the solvents used. As LHSS was prepared in stock solutions, its concentration varied depending on the volume of the LHSS stock solution included in the sample. All samples were sealed using Parafilm® and shaken at 800 rpm on an orbital shaker (VWR, Leicestershire, UK) set at 32 °C for 24 h. The samples were kept at room temperature and assessed at 24 h and 72 h.

Stability testing of formulations

Prior to use in IVPT, the stability of all formulations was evaluated for 72 h. Stability testing was undertaken using the method previously reported by the authors.[13] Any formulations where precipitation was visually detected, were disregarded. Where no precipitation was apparent, samples were analysed using HPLC.

Solubility parameters (SP) of solvents

SPs of single solvents were determined previously[13] using the Van Krevelen and Hoftyzer method, incorporated within the Molecular Modelling Pro software, version 7.0.8 (Norgwyn Montgomery Software Inc., Pennsylvania, USA). The calculation of SP values for binary or ternary solvents were based on the volume fraction of the solvent as shown in eqn (1):[16–18]

$$(\delta)^n = \frac{(\delta^i \times \Phi^i) + (\delta^j \times \Phi^j) + (\delta^k \times \Phi^k)}{(\Phi^i + \Phi^j + \Phi^k)} \quad (1)$$

where $(\delta)^n$ denotes the SP of the solvent mixture, δ^i, δ^j and δ^k represent the SP of the individual solvents, and Φ^i, Φ^j and Φ^k refers to the volume of each solvent.

Finite dose (10 μL) porcine skin IVPT and mass balance studies

IVPT and mass balance studies were conducted as previously reported.[12,13] IVPT was conducted using vertical glass Franz diffusion cells (Soham Scientific, Cambridgeshire, UK), with receptor medium consisting of 6% w/v Brij™ O20 in phosphate-buffered saline (pH 7.3 ± 0.2) to maintain sink conditions. Diffusion cells were placed in a Grant Sub Aqua 26 water bath (Grant Instruments, Cambridgeshire, UK) preheated to ~37 °C to achieve a skin surface temperature of 32 ± 1 °C. Finite doses (10 μL) of formulations were applied. Final samples were collected at 25 h. Membranes were washed three times and extracted with a mixture of methanol and water (85 : 15 v/v).

Data analysis

The mean and standard deviation (SD) of the data was calculated using Microsoft Excel® version 16.94 (Microsoft Corporation, Washington, U.S.). IBM® SPSS Statistics® Version 29.0.1.1 (IBM, New York, US) was used to carry out further statistical analysis. The normality of distribution of the data sets was evaluated using the Shapiro–Wilk test. For parametric data, statistical significance was analysed using a one-way analysis of variance (ANOVA), combined with Tukey's *post hoc* test. Where only two samples were compared, the independent-samples *t*-test was used. Statistical significance of non-parametric data was assessed using the Kruskal–Wallis one-way ANOVA (*k*-samples) with multiple pairwise-comparisons where there were more than two samples. Alternatively, for two samples, the Mann–Whitney *U* test was used. Probability values where $p < 0.05$ were considered statistically significant.

Results and discussion

Binary solvents: effect of solvent substitution

The formulations applied comprised DiPG and water (60 : 40 v/v) and contained 10 mg mL^{-1} DNa and either 10 or 0 mg mL^{-1} LHSS. To assess the influence of alternative non-aqueous solvents on the percutaneous delivery of DF, a study involving solvent substitution was conducted. Earlier work had identified, but not tested, a binary system containing 60% DiPG and 40% water (v/v) with 10 mg mL^{-1} DNa and either 0 or 10 mg mL^{-1} LHSS.[13] As this system corresponded to a previously evaluated formulation with 60% TC and 40% water (v/v) at the same DNa and LHSS concentrations (10 mg mL^{-1} DNa; 10 or 0 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v), it was selected to determine the impact of solvent substitution.

Table 1 and Fig. 1 show that the addition of LHSS at 10 mg mL^{-1} to the formulation containing 10 mg mL^{-1} DNa and comprising DiPG : water (60 : 40 v/v), resulted in a DF permeation value of 0.53 ± 0.34 μg cm^{-2}, amounting to 0.62 ± 0.42% of the applied dose. While this was higher than the amount of DF permeating from the DiPG : water (60 : 40 v/v) control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v) at 0.14 ± 0.28 μg cm^{-2} (0.16 ± 0.32%), the difference was not statistically significant ($p > 0.05$). As reported in Table S1, permeation values for the corresponding TC : water (60 : 40, v/v) study, were 1.01 ± 0.91 μg cm^{-2} for the LHSS containing formulation (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v) and 0.36 ± 0.44 μg cm^{-2} for the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v). These results were determined to be comparable to the DiPG : water (60 : 40, v/v) permeation values ($p > 0.05$).

The percentage of DF extracted from the membrane, however, was significantly higher for the DiPG : water (60 : 40, v/v) formulation containing LHSS (2.07 ± 0.71%, 10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v), than for the DiPG : water (60 : 40, v/v) control formulation (0.87 ± 0.23%, 10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v, $p < 0.05$). Nonetheless, when compared to the TC : water (60 : 40,

Table 1 Results for finite dose (10 μL) porcine skin IVPT. Formulations were preparedwith DiPG and water (60 : 40 v/v); 10 mg mL^{-1} DNa; and 0 or 10 mg mL^{-1} LHSS (n = 5; mean ± SD)

	DiPG : water (60 : 40 v/v)	
Amount DF retained in the membrane and permeated	DNa 10 mg mL^{-1} : LHSS 10 mg mL^{-1}	DNa 10 mg mL^{-1} : LHSS 0 mg mL^{-1}
Cumulative permeation μg cm^{-2} at 25 h	0.53 ± 0.34	0.14 ± 0.28
Permeated 25 h %	0.62 ± 0.42	0.16 ± 0.32
Retained on skin surface %	99.86 ± 3.84	101.38 ± 6.41
Retained in membrane %	2.07 ± 0.71	0.87 ± 0.23
Retained in membrane plus permeated %	2.69 ± 1.01	1.02 ± 0.44
Recovery %	102.55 ± 3.32	102.41 ± 6.10
DNa : LHSS molar ratio	1 : 1.41	1 : 0

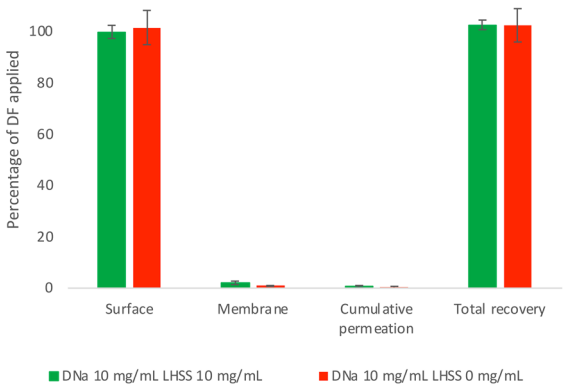

Fig. 1 Percentage recovery (mean ± SD) of DF from mass balance studies, following porcine IVPT. Finite doses (10 μL) of the binary solvent formulations prepared with DiPG and water (60 : 40 v/v), containing 10 mg mL^{-1} DNa and 0 or 10 mg mL^{-1} LHSS, were applied (n = 5; mean ± SD).

v/v) system, it was determined that the percentages of DF extracted from both the LHSS-containing formulation (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v, 4.31 ± 1.34%) and the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v, 4.39 ± 0.95%), were significantly greater ($p < 0.05$).

The total percentage of DF applied that was retained in the membrane and permeated from the DiPG : water (60 : 40, v/v) system was significantly higher when the counterion was included (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v), 2.69 ± 1.01%, relative to the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v), 1.02 ± 0.44% ($p < 0.05$). Similarly, these values were significantly lower than those observed for the TC : water solvent system, where the inclusion of LHSS (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v) resulted in a total percentage of 5.41 ± 2.21% and the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v) 4.80 ± 1.08% ($p < 0.05$).

Thus the incorporation of the counterion in the DiPG : water (60 : 40, v/v) formulations (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v) significantly increased the total percentage of DF that moved into and through the skin, when compared to the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v). Conversely, when examining the equivalent TC : water (60 : 40, v/v) formulations, the addition of LHSS (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v) had no significant effect on the total percentage of DF that was extracted from the membrane and permeated. Despite this, the TC : water (60 : 40, v/v) formulations, whether including or excluding LHSS, resulted in significantly higher percentages of DF that was retained in the membrane and permeated, than the DiPG : water (60 : 40, v/v) formulations.

An initial analysis of the SPs and their effects on the solute's thermodynamic activity in the solvent system, did not entirely account for the observed results. The calculated[19] and experimentally determined[13] SP for the active (22.65 $MPa^{1/2}$) is more closely aligned to that of the TC : water binary solvent system (31.83 $MPa^{1/2}$) than the DiPG : water system (34.73 $MPa^{1/2}$). Thus, the DiPG : water system would be expected to facilitate greater movement of the active into the skin due to a higher thermodynamic activity of the solute in the solvent system.[20] However, this was not supported by the experimental data. One potential explanation for this discrepancy is that treating the solvent system as a uniform whole overlooks the influence of individual excipients, which may have their own effects.[21] When examined separately, DiPG has a solubility parameter (SP) of 26.54 $MPa^{1/2}$, while TC has an SP of 21.72 $MPa^{1/2}$. The value for TC is similar to both the amount estimated for the skin (20.46 $MPa^{1/2}$)[22] and that determined for the active. Moreover, evidence from various finite-dose IVPT using multiple formulations containing TC as an excipient, supports this idea. In these studies approximately 40–60% of the TC included in the applications was either extracted from the membrane or permeated.[23] As the total recovery of TC was never greater than 1% more than the amount that had partitioned and permeated, the studies were repeated under occlusive conditions. The total recovery of TC increased to between 85 and 93%, with the loss attributed to evaporation.[23] Published data using dynamic vapour sorption (DVS)[24–28] and IVPT studies[24,28] confirm that TC[25–27] evaporates more rapidly than DiPG[25] due to its higher vapour pressure,[29,30] resulting in an overall lower recovery. Consequently, TC primarily evaporates or penetrates into the skin, whereas DiPG with its lower volatility, remains largely recoverable from the surface.[26] As TC leaves the formulation, whether by skin absorption or evaporation, the relative water content increases. Since the drug is only sparingly soluble in water,[14] this shift enhances its thermodynamic activity,[14,18,31–34] thereby promoting drug penetration into the skin.[34]

Additionally, it has been suggested that TC's lower dielectric constant, 14.10 at 25 °C,[35] in contrast to DiPG's higher value, 19.80 at 25 °C,[36] suppresses the complete ionisation of salts by promoting ion pairing within the salts themselves.[37] This might reduce the need for the ion pair, LHSS, and could partially explain why there was no significant difference between the TC formulations that contained LHSS and the control (absence of LHSS).

In addition to TC's influence on charge reduction and its impact on the thermodynamic driving force of the active, it has been suggested that TC also acts upon the SC in various ways.[37] It has been associated with an increase in the solubility of actives in the stratum corneum,[38] and has been shown to interact with several SC proteins and lipids, potentially increasing their mobility.[39] The increased mobility of ceramide headgroups has been linked to a disruption in the packing of the interfacial headgroup regions of the lipid layers.[39] Such disruptions have been associated with an increase in the diffusion of active ingredients.[40] However, such published mechanistic studies do not represent finite dose applications but rather reflect the impact on the SC under saturated conditions.[38,39] Nonetheless, a number of publications that have considered

the distribution of excipients, in addition to the active pharmaceutical ingredient (API), have reported that the permeation of the API has closely followed the permeation of TC in both single[24,27,41] and binary[27] solvent systems.

In the present study, the percentage recovery of the DF applied was within the range recommended by the OECD guidelines,[42] at 102.55 ± 3.32% for the LHSS-containing formulation (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v) and 102.41 ± 6.10% for the control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v).

Ternary DNa–LHSS loaded miscibility studies

The three solvents in which DNa was most soluble, were selected for use in ternary DNa–LHSS miscibility tests. As DNa was most soluble in TC, non-aqueous fractions comprised TC with either DiPG or PG.

DiPG : TC : water (10 : 40 : 50 v/v/v) combinations contained DNa at 7.5 and 5 mg mL^{-1}, and L-HSS at 12.5 or 0 mg mL^{-1}.

Alternative ternary solvent systems comprised PG : TC : water (10 : 40 : 50 v/v/v) and included DNa at 5 mg mL^{-1} and L-HSS at either 25 mg mL^{-1}, 12.5 or 0 mg mL^{-1}. The selected ternary systems were miscible and had no visible precipitation.

Finite dose (10 μL) ternary IVPT and mass balance studies

Ternary solvents DiPG : TC : water (10 : 40 : 50 v/v/v) containing 7.5 mg mL^{-1} DNa and either 12.5 or 0 mg mL^{-1} LHSS. As shown in Table 2 the addition of LHSS (7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) resulted in an increase of approximately 36% in the permeation of DF (1.48 ± 1.13 μg cm^{-2} or 2.20 ± 1.68% of DF applied) when compared to the control formulation (1.09 ± 0.82 μg cm^{-2} or 1.63 ± 1.22% of DF applied, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v). This increase was not considered statistically significant ($p > 0.05$). Furthermore, these results were considered comparable to binary permeation experiments using 7.5 mg mL^{-1} DNa and combining TC and water (50 : 50 v/v, $p > 0.05$). As shown in Table S1, the LHSS-containing TC : water binary formulations (7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) resulted in a permeation of 1.49 ± 0.75 μg cm^{-2} (2.24% of the DF applied), while the formulation without LHSS (7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) showed a permeation of 0.22 ± 0.19 μg cm^{-2} (0.35% of the DF applied, $p > 0.05$).

Similarly, the percentages of DF extracted from the membrane were comparable for the ternary formulation containing LHSS (7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v), 6.37 ± 3.14% and the ternary control (7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v), 5.27 ± 1.37% ($p > 0.05$). No significant differences were observed between LHSS-containing binary (8.14 ± 2.24%, 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) and ternary (6.37 ± 3.14%, 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) formulations ($p > 0.05$), or binary (3.95 ± 0.12%, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) and ternary (5.27 ± 1.37%, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) control formulations ($p > 0.05$).

Likewise, the ternary formulation with LHSS (9.66 ± 5.77%, 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) was comparable to the ternary control (7.83 ± 3.67%, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v, $p > 0.05$). This extended to comparisons between the binary (10.38 ± 2.49%, 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) and ternary (9.66 ± 5.77%, 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) formulations containing the ion pair ($p > 0.05$). Similarly, the binary (4.30 ± 0.42%, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) and ternary (7.83 ± 3.67%, 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) control formulations were also comparable ($p > 0.05$).

The recovery of DF conformed with recommendations outlined in the OECD guidelines.[42] This can be seen in both Table 2 and Fig. 2 representing mass balance results, which indicate that the recovery of DF was 98.05 ± 1.60% (7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) and 100.84 ± 2.43% (7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) respectively.

Ternary solvents DiPG : TC : water (10 : 40 : 50 v/v/v), containing 5 mg mL^{-1} DNa and either 12.5 or 0 mg mL^{-1} LHSS. Although the decrease in concentration of DNa resulted in an increase in the DNa : LHSS molar ratio from 1: 2.35 to 1: 3.5, there was no significant difference in the total percentage of DF

Table 2 Results for finite dose (10 μL) porcine IVPT. Ternary solvent formulations were prepared with DiPG : TC : water (10 : 40 : 50 v/v/v), 7.5 mg mL^{-1} DNa and 0 or 12.5 mg mL^{-1} LHSS (n = 5; mean ± SD)

	DiPG : TC : water (10 : 40 : 50 v/v/v)	
Amount DF retained in the membrane and permeated	DNa 7.5 mg mL^{-1} :LHSS 12.5 mg mL^{-1}	DNa 7.5 mg mL^{-1} : LHSS 0 mg mL^{-1}
Cumulative permeation μg cm^{-2} at 25 h	1.48 ± 1.13	1.09 ± 0.82
Permeated 25 h %	2.20 ± 1.68	1.63 ± 1.22
Retained on skin surface %	90.87 ± 2.51	94.45 ± 1.40
Retained in membrane %	4.98 ± 0.61	4.76 ± 0.89
Retained in membrane plus permeated %	7.18 ± 1.86	6.39 ± 2.02
Recovery %	98.05 ± 1.60	100.84 ± 2.43
DNa : LHSS molar ratio	1 : 2.35	1 : 0

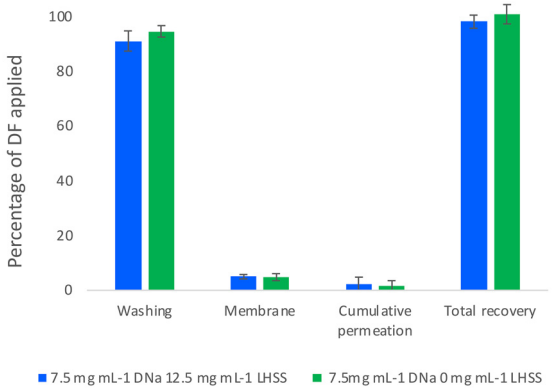

Fig. 2 Percentage recovery (mean ± SD) of DF from mass balance studies, following porcine IVPT. Finite doses (10 μL) of the ternary solvent formulations prepared with DiPG : TC : water (10 : 40 : 50 v/v/v), containing 7.5 mg mL^{-1} DNa and 0 or 12.5 mg mL^{-1} LHSS (n = 5; mean ± SD).

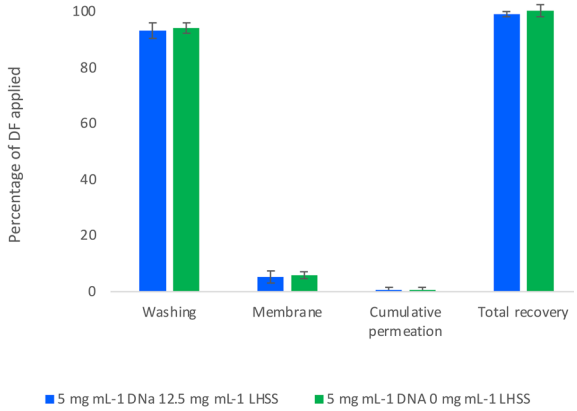

Fig. 3 Percentage recovery (mean ± SD) of DF from mass balance studies, following porcine IVPT. Finite doses (10 μL) of the ternary solvent formulations were prepared with DiPG : TC : water (10 : 40 : 50 v/v/v), containing 5 mg mL^{-1} DNa and 0 or 12.5 mg mL^{-1} LHSS ($4 \leq n \leq 5$; mean ± SD).

that was extracted from the membrane and permeated for the 5 and 7.5 mg mL^{-1} DiPG-containing ternary samples ($p > 0.05$).

As shown in Table 3 and Fig. 3 the cumulative permeation of DF for the LHSS-containing ternary formulation with 5 mg mL^{-1} DNa (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) was 0.21 ± 0.42 μg cm^{-2}. This value was almost identical to that of the corresponding ternary control formulation (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) which measured 0.21 ± 0.36 μg cm^{-2}. These amounts represented 0.47 ± 0.93% and 0.48 ± 0.80% of the DF applied, respectively, with no significant difference between them ($p > 0.05$). As shown in Table S1, the binary formulation containing LHSS (1.48 ± 0.65 μg cm^{-2}, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) resulted in significantly higher permeation values for DF than the equivalent ternary formulation (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v, $p < 0.05$). However, the results for the control formulations (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, 0.79 ± 0.62 μg cm^{-2} and 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) were comparable ($p > 0.05$).

When examining the percentage of DF extracted from the membrane, no significant differences were detected between the ternary formulation containing LHSS (5.15 ± 1.99%, DiPG : TC : water; 10 : 40 : 50; v/v/v) and the corresponding ternary control formulation (5.71 ± 1.17%, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v). However, the percentage of DF retained in the membranes of both ternary formulations (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v and 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) was significantly lower than that of the equivalent binary formulations (8.79 ± 2.05%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v and 7.60 ± 1.19%, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, $p < 0.05$).

As with the previous results, the total DF value for the ternary sample containing the ion pair (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) was 5.62 ± 2.78%, was comparable to that of the ternary control formulation (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) which had a value of 6.20 ± 1.60% ($p > 0.05$). Similarly, the total DF value for the ternary control formulation (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) was similar to that of the binary control formulation (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v), which resulted in 9.36 ± 2.49% ($p > 0.05$). However, when LHSS was included,

Table 3 Results for the finite dose (10 μL) porcine IVPT. Ternary solvent formulations prepared with DiPG : TC : water (10 : 40 : 50 v/v/v), 5 mg mL^{-1} DNa and 0 or 12.5 mg mL^{-1} LHSS. ($4 \leq n \leq 5$; mean ± SD)

	DiPG : TC : water (10 : 40 : 50 v/v/v)	
Amount DF retained in the membrane and permeated	DNa 5 mg mL^{-1} : LHSS 12.5 mg mL^{-1}	DNa 5 mg mL^{-1} : LHSS 0 mg mL^{-1}
Cumulative permeation μg cm^{-2} at 25 h	0.21 ± 0.42	0.21 ± 0.36
Permeated 25 h %	0.47 ± 0.93	0.48 ± 0.80
Retained on skin surface %	93.49 ± 5.03	93.99 ± 1.75
Retained in membrane %	5.15 ± 1.99	5.71 ± 1.17
Retained in membrane plus permeated %	5.62 ± 2.78	6.20 ± 1.60
Recovery %	99.11 ± 2.31	100.19 ± 1.87
DNa : LHSS molar ratio	1 : 3.5	1 : 0

the total DF value for the ternary formulation (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) was significantly lower than that observed for the binary formulation (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v) which reached 12.26 ± 3.06% ($p < 0.05$).

Similar to the 7.5 mg mL^{-1} formulations, no statistically significant differences were observed between the ternary samples ($p > 0.05$). However, the impact of TC replacement was more evident in the 5 mg mL^{-1} samples. The inclusion of DiPG significantly reduced the total amount of DF extracted and permeated in the ternary formulation containing LHSS (5.62 ± 2.78%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) compared to the corresponding binary formulation (12.26 ± 3.06%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, $p < 0.05$).

The recovery of DF was consistent with OECD guidelines,[42] with 99.11 ± 2.31% recovered for the LHSS-containing preparation (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v) and 100.19 ± 1.87% for the control (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v).

Ternary solvents PG : TC : water (10 : 40 : 50 v/v), containing 5 mg mL^{-1} DNa and either 25, 12.5 or 0 mg mL^{-1} LHSS. The final ternary system studied maintained the concentration of DNa at 5 mg mL^{-1}, while varying the LHSS concentration across the three sample types: 25 mg mL^{-1}, 12.5 mg mL^{-1} and a control group with no LHSS. The primary modification involved replacing DiPG with PG, resulting in a solvent mixture of PG : TC : water (10 : 40 : 50 v/v/v).

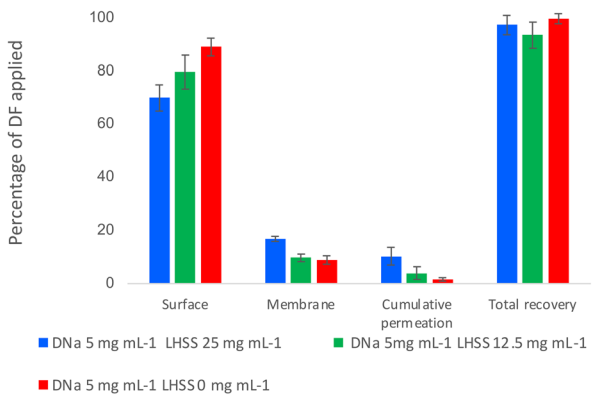

Fig. 4 Percentage recovery (mean ± SD) of DF from mass balance studies, following porcine IVPT using 10 μL of the ternary solvent formulations prepared with PG : TC : water (10 : 40 : 50 v/v/v), containing 5 mg mL^{-1} DNa and 0, 12.5 or 25 mg mL^{-1} LHSS ($4 \leq n \leq 5$; mean ± SD).

Fig. 4 and Table 4 summarise the cumulative permeation data for DF, as well as the results of mass balance investigations. The cumulative permeation of DF from samples containing 25 mg mL^{-1} LHSS (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) amounted to 4.26 ± 1.41 μg cm^{-2}. This was significantly greater ($p < 0.05$) than the permeation from samples containing 12.5 mg mL^{-1} LHSS (1.72 ± 1.06 μg cm^{-2}, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) and the control samples (0.66 ± 0.30 μg cm^{-2}, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v), which were comparable ($p > 0.05$). These permeation amounts corresponded to 10.33 ± 3.27% (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v), 3.95 ± 2.43% (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) and 1.51 ± 0.69% (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) of the DF applied.

The DF permeation from the PG-containing ternary formulation comprising LHSS at 25 mg mL^{-1} (4.26 ± 1.41 μg cm^{-2}, 5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) was significantly greater than that from any of the 5 mg mL^{-1}, 7.5 mg mL^{-1} and 10 mg mL^{-1} DNa formulations, whether binary (0.14 ± 0.28 – 1.52 ± 0.32 μg cm^{-2}) or ternary (0.21 ± 0.36 – 1.72 ± 1.06 μg cm^{-2}) solvent systems were used.

Furthermore, this ternary formulation (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) resulted in approximately 2.5 times (145% more) the DF permeation of a commercial 1% DNa formulation (1.74 ± 0.6 μg cm^{-2} at 24 h) under finite dose conditions, as shown in Table 5. Notably, this was observed despite containing only half the active concentration of the commercial formulation. When expressed as a percentage of the DF applied, permeation from the ternary formulation (10.33 ± 3.27%, 5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) was significantly greater than that from the commercial formulation (2.10 ± 0.72%, $p < 0.05$).

Table 4 Results for the finite dose (10 μL) porcine IVPT. Ternary solvent formulations prepared with PG : TC : water (10 : 40 : 50 v/v/v), 5 mg mL^{-1} DNa and 0, 12.5 or 25 mg mL LHSS and ($4 \leq n \leq 5$; mean ± SD)

	PG : TC : water (10 : 40 : 50 v/v/v)		
Amount DF retained in the membrane and permeated	DNa 5 mg mL^{-1} : LHSS 25 mg mL^{-1}	DNa 5 mg mL^{-1} : LHSS 12.5 mg mL^{-1}	DNa 5 mg mL^{-1} : LHSS 0 mg mL^{-1}
Cumulative permeation μg cm^{-2} at 25 h	4.26 ± 1.41	1.72 ± 1.06	0.66 ± 0.30
Permeated 25 h %	10.33 ± 3.27	3.95 ± 2.43	1.51 ± 0.69
Retained on skin surface %	69.91 ± 4.92	79.53 ± 6.24	88.99 ± 4.21
Retained in membrane %	16.92 ± 1.04	9.87 ± 1.46	8.96 ± 1.49
Retained in membrane plus permeated %	27.25 ± 2.61	13.82 ± 3.57	10.47 ± 2.09
Recovery %	97.16 ± 3.51	93.35 ± 4.76	99.47 ± 2.74
DNa : LHSS molar ratio	1 : 7.1	1 : 3.5	1 : 0

Table 5 Results for the finite dose (10 μL) porcine IVPT using the commercial formulation containing 10 mg mL^{-1} DNa (n = 4; mean ± SD)

Amount DF retained in the membrane and permeated	DNa 10 mg mL^{-1}
Cumulative permeation μg cm^{-2} at 24 h	1.74 ± 0.60
Permeated 24 h %	2.10 ± 0.72
Retained on skin surface %	69.91 ± 4.92
Retained in membrane %	6.06 ± 0.67
Retained in membrane plus permeated %	8.20 ± 1.37
Recovery %	102.74 ± 4.23

No significant difference was observed in DF permeation between the PG-ternary formulations containing 12.5 mg mL^{-1} LHSS (1.72 ± 1.06 μg cm^{-2}, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) or no LHSS (0.66 ± 0.30 μg cm^{-2}, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) and the equivalent 5 mg mL^{-1} DNa DiPG-ternary formulations (0.21 ± 0.42 μg cm^{-2}, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v and 0.21 ± 0.36 μg cm^{-2}, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v $p > 0.05$).

Membrane retention of DF was 16.92 ± 1.04%, 9.87 ± 1.46% and 8.96 ± 1.49% of the DF applied for PG-containing ternary samples with 25 mg mL^{-1}, 12.5 mg mL^{-1} and 0 mg mL^{-1} LHSS respectively. The total percentage of DF recovered through extraction from the membrane and permeation was significantly higher for the 25 mg mL^{-1} LHSS formulation (27.25 ± 2.61%, 5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v, $p < 0.05$) than for the 12.5 mg mL^{-1} LHSS (13.82 ± 3.57%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) and control (10.47 ± 2.09%, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) formulations, which were comparable ($p > 0.05$).

The percentage of DF extracted from the membrane, as well as the total percentage of DF retained in the membrane and permeated, were significantly greater for the PG ternary formulation containing 25 mg mL^{-1} LHSS (16.92 ± 1.04% and 27.25 ± 2.61%, 5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) than for other binary and ternary formulations (0.87 ± 0.23% – 8.79 ± 2.05% and 1.02 ± 0.44% – 12.26 ± 3.06%), with only one exception. The binary TC : water formulation also containing 25 mg mL^{-1} LHSS (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v), was considered comparable for these values (11.00 ± 7.21% and 14.49 ± 7.76%, $p > 0.05$).

When compared to the commercial formulation, the PG ternary formulation (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) showed significantly greater membrane retention (16.92 ± 1.04% *vs.* 6.06 ± 0.67%) and total DF extracted from the membrane and permeated (27.25 ± 2.61% *vs.* 8.20 ± 1.37%, $p < 0.01$).

Furthermore, the percentages of DF retained within the membrane, and the total DF retained plus permeated, were significantly greater from the PG-ternary formulations with either 12.5 mg mL^{-1} LHSS (9.87 ± 1.46% and 13.82 ± 3.57%, 5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) or no LHSS (8.96 ± 1.49% and 10.47 ± 2.09%, 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) when compared to other DiPG-ternary (5.62 ± 2.78%–7.18 ± 1.86%) systems ($p < 0.05$). However, no significant difference was observed when comparing these PG-ternary formulations (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v and 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) with TC : water (50 : 50 v/v) binary samples (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v and 5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v), in which case the values ranged from 8.14 ± 2.24% – 11.00 ± 7.21% for membrane retention, and 10.38 ± 2.49% – 14.49 ± 7.76% for total DF recovery from membrane retention and permeation ($p > 0.05$).

The replacement of 10% (v/v) DiPG with 10% (v/v) PG had significant effects on the amounts of DF retained in the membrane, as well as the combined amounts attributable to membrane extraction and permeation ($p < 0.05$).

While the impact of the dielectric constant values of solvents should always be considered when investigating ion pair behaviour,[43,44] the replacement of DiPG[36] with PG[45,46] would be unlikely to facilitate ion pairing due to its higher value. Furthermore, while the slightly higher SP value for the PG-ternary system (35.06 MPa$^{1/2}$), relative to the DiPG-ternary system (34.84 MPa$^{1/2}$), may contribute to the increased uptake of DF, analysis of the individual solvents suggests a more complex account. Notwithstanding its SP of 28 MPa$^{1/2}$, PG was found to be a more effective solubiliser for the active (SP 22.65 MPa$^{1/2}$)[13,19] than DiPG, which has a SP value of 26.54 MPa$^{1/2}$. This higher affinity should correspond to a reduction in the thermodynamic activity of the active in the solvent system.[20] However, although the skin's SP[22] is more closely aligned with DiPG than PG, finite dose studies investigating excipient behaviour produced results contrary to these numerical predictions. Specifically, single-solvent studies showed that 98.9% of DiPG remained on the skin surface after 48 hours, compared to less than 7% of PG.[24] While this result may be attributed in part to evaporation, due to PG's higher vapour pressure[47] relative to DiPG's,[30] the quantities of solvent that moved into and through the membrane suggested a greater affinity between PG and the skin than between DiPG and the skin.[24] This movement of PG into the skin combined with possible evaporation could contribute to an increase in the water portion of the solvent system. As DF is sparingly soluble in water[14] this would enhance its thermodynamic activity in the changing vehicle, and promote its rate of penetration into the SC.

The mechanism of action of PG has been widely debated. It has been suggested that it enhances drug solubility in the skin,[48–50] influences partitioning behaviour of actives,[49,50] and disrupts the barrier properties of the SC under occluded infinite dose conditions.[51]

It has also been proposed that PG facilitates the movement of drugs through the skin by solvating alpha-keratin and inhabiting hydrogen-bonding sites, resulting in a reduction to

drug-membrane bonds.[52] Differential scanning calorimetry (DSC) studies in human skin suggest that PG behaves like water, forming hydrogen-bonds with polar head groups. This results in a loosening of the lipid packing and a reduction in intermolecular forces, potentially facilitating drug migration.[52] Findings on PG's impact on lipid organisation remain inconsistent. Bouwstra *et al.*[53] proposed that PG and water integrate into polar head group regions without altering bilayer spacing, while Brinkman and Müller-Goymann observed vertical and horizontal integration, resulting in an increase in the distance between the repeating bilayers.[54] In contrast, Moghadam and colleagues found that PG resulted in no structural changes to SC lipids.[55] More recently, however, Synchrotron-Based Fourier Transform Infrared Spectroscopy indicated that PG resulted in alterations to the bilayer structure of intercellular lipids, with disorder occurring on an increasing basis from the stratum corneum to the deeper regions of the viable epidermis and the dermis.[56]

Despite the variable explanations for its mechanism of action, PG is widely used in topical formulations, with percutaneous absorption frequently reported.[24,28,57–60] It has been used to increase the solubility of actives and as a permeation enhancer.[26,27,48,52,56,61–65] It has also been proposed as a "carrier solvent", provided the PG was able to partition out of the formulation and into the skin.[57]

Various infinite[49,50,66] and finite dose[61] studies using human skin have reported correlations between PG application and drug permeation. This has been confirmed using confocal Raman spectroscopy (CRS).[67,68]

When PG was combined with water in infinite dose *in vivo*[49] and *in vitro*[50,69] applications, the permeation of various compounds were shown to increase with rising concentrations of PG. Furthermore, PG has demonstrated synergistic effects when combined with other permeation enhancers such as terpenes,[70] fatty acids[66] and amides.[52] It is unsurprising therefore that the combination of PG and TC has been reported to enhance the uptake of active pharmaceutical ingredients, when compared to the individual solvents, in finite dose applications.[27,65,71] PG, which permeates more gradually than TC,[24] has also been shown to augment the permeation of TC.[27] Our studies demonstrate the significant increase in the total percentage of DF moving into and through the membrane for all ternary samples containing PG (PG : TC : water; 10 : 40 : 50; v/v/v) relative to all binary and ternary formulations containing DiPG (DiPG : water; 60 : 40; v/v and DiPG : TC : water; 10 : 40 : 50; v/v/v).

The synergistic behaviour of TC and PG also helps to explain the increase in permeation of DF from the ternary formulation containing PG and LHSS at 25 mg mL^{-1} LHSS (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) relative to the binary formulation that contained no PG (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v).

As shown in Fig. 4, recovery of DF through mass balance studies was within the guidelines set by the OECD.[42] The amount of DF was 97.16 ± 3.51% for the 25 mg mL^{-1} LHSS samples (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v), 93.35 ± 4.76% for the 12.5 mg mL^{-1} LHSS samples (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) and 99.47 ± 2.74% for the control samples (5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v).

Conclusions

The aim of this study was to continue the investigation into the impact of solvents on DNa : LHSS ion pair formulations. Replacing TC with DiPG resulted in a binary solvent system comprising DiPG and water (60 : 40 v/v). While the inclusion of the counterion in the DiPG formulation (10 mg mL^{-1} DNa; 10 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v) significantly enhanced the total percentage of DF that passed into and through the skin, when compared to the DiPG control (10 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : water; 60 : 40; v/v), the solvent substitution significantly reduced the membrane retention and total DF extracted from the membrane and permeated when compared to the original TC formulations (10 mg mL^{-1} DNa; 10 mg mL^{-1} or 0 mg mL^{-1} LHSS; TC : water; 60 : 40; v/v, $p < 0.05$).

The investigation into the use of ternary systems produced further insights. When considering the 7.5 mg mL^{-1} DNa formulations, the inclusion of DiPG had no significant effect on the movement of DF into and through porcine skin, whether comparing L-HSS-containing ternary and binary preparations (7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v and 7.5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, $p > 0.05$) or their controls (7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v and 7.5 mg mL^{-1} DNa; 0 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, $p > 0.05$).

For the ternary system comprising 5 mg mL^{-1} DNa, the effects of replacing TC with DiPG were more apparent. At this concentration of DNa, amounts of DF moving into and through the membrane from the LHSS-containing ternary formulation (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; DiPG : TC : water; 10 : 40 : 50; v/v/v) were significantly less than from the binary TC : water formulation containing the counterion (5 mg mL^{-1} DNa; 12.5 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v, $p < 0.05$).

In contrast to DiPG, which exhibited some limiting effects on the percutaneous delivery of DF, its substitution with PG (10% v/v), resulted in significant improvements. These included higher percentages of DF retained in the membrane and increased total values attributed to membrane extraction and permeation, compared to all DiPG binary and ternary formulations ($p < 0.05$).

Moreover, the addition of LHSS in the ternary formulation containing PG (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; PG : TC : water; 10 : 40 : 50; v/v/v) resulted in significantly higher DF permeation values than any other binary or ternary formulation ($p < 0.05$). It also delivered approximately 2.5 times (145%) more DF when compared to a commercial 1%

DNa formulation, despite containing only half the DNa concentration.

Additionally, the PG ternary formulation containing 25 mg mL^{-1} LHSS produced the highest total DF total uptake as a percentage of the applied dose ($p < 0.05$), comparable only to the one other preparation which contained LHSS at the same 25 mg mL^{-1} concentration (5 mg mL^{-1} DNa; 25 mg mL^{-1} LHSS; TC : water; 50 : 50; v/v).

When examining the impact of solvent substitutions in formulations containing TC and DiPG, the analysis of individual solvents revealed complexities beyond those predicted by the SP of the overall solvent system. Such an approach would overlook the influence of individual excipients, which may exert their own effects. Instead, when evaluated separately, TC has a SP more closely aligned with DNa and the skin, than DiPG.

A similar approach was required when analysing PG and DiPG in ternary systems. Despite PG's lower predicted affinity for both the active and the skin relative to DiPG, PG proved to be a more effective solvent for DNa. Like TC, PG's ability to penetrate into the skin coupled with its higher volatility relative to DiPG, may contribute to an increase in the water content of the system. This in turn would increase the thermodynamic activity of DNa in the changing vehicle, as it is sparingly soluble in water, thereby increasing its rate of penetration into the SC.

An assessment of dielectric constant values showed that TC's lower dielectric constant is more effective at both suppressing ionisation and promoting ion pairing, than DiPG. In contrast, while PG has a higher dielectric constant value than DiPG, any influence in the ternary system may be limited by its relatively low proportion in the solvent composition (10% v/v).

This work demonstrates how passive enhancement methods, such as counterion use and solvent selection, can be effectively combined to improve the percutaneous delivery of a topically applied pharmaceutical salt. A non-toxic, economical, and sustainably produced counterion was identified, which significantly increased DF penetration. The role of various solvents in maximising the solubility of the API and counterion was also explored, focusing on their potential as permeation enhancers and their impact on the thermodynamic activity of the API within formulations.

Using a commercial formulation as a benchmark suggests that this approach could reduce the required API concentration in formulations, offering both economic and environmental advantages. Future work could involve optimising the current formulations by experimenting with additional solvents to improve the solubility and stability of the API within the formulation. Supplementary adjustments to the concentration of LHSS could further enhance the delivery of the API. In addition, this approach could serve as a versatile framework for other topically applied NSAIDs. This includes APIs formulated as salts (*e.g.*, ketorolac tromethamine) or those that undergo ionisation, expanding the potential applications of the method to a wider range of drugs with similar delivery challenges.

Author contributions

Mignon Cristofoli: conceptualization, methodology, validation, investigation, formal analysis, visualization, writing – original draft. Jonathan Hadgraft: conceptualization. Majella E. Lane: conceptualization, resources, supervision, writing – review & editing. Bruno C. Sil: conceptualization, resources, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

ACN	Acetonitrile
API	Active pharmaceutical ingredient
ANOVA	Analysis of variance
DF	Diclofenac
DNa	Diclofenac sodium
DSC	Differential scanning calorimetry
DiPG	Dipropylene glycol
DVS	Dynamic vapour sorption
LHSS	L-Histidine monochloride monohydrate
ICH	International conference on harmonisation expert working group
IVPT	*In vitro* permeation testing
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
PBS	Phosphate buffered saline
PG	Propylene glycol
SP	Solubility parameters
SD	Standard deviation
TC	Transcutol®
TFA	Trifluoroacetic acid

Data availability

The data supporting this article have been included as part of the SI.

Supplementary information comprises 3 parts. Part (i) raw data, part (ii) data relating to previously published IVPT (binary solvent formulations) and (iii) confirmation of ion pairs using FT-IT Spectroscopy. See DOI: **https://doi.org/10.1039/d5pm00096c**.

References

1 D. J. Hunter, L. March and M. Chew, *Lancet*, 2020, **396**, 1711–1712.
2 V. P. Leifer, J. N. Katz and E. Losina, *Osteoarthr. Cartil.*, 2022, **30**, 10–16.
3 G. A. Brown, *J. Am. Acad. Orthop. Surg.*, 2013, **21**, 577–579.

4 R. R. Bannuru, M. Osani, E. Vaysbrot, N. Arden, K. Bennell, S. Bierma-Zeinstra, V. Kraus, L. S. Lohmander, J. Abbott and M. Bhandari, *Osteoarthr. Cartil.*, 2019, **27**, 1578–1589.
5 O. Bruyère, G. Honvo, N. Veronese, N. K. Arden, J. Branco, E. M. Curtis, N. M. Al-Daghri, G. Herrero-Beaumont, J. Martel-Pelletier and J.-P. Pelletier, *Semin. Arthritis Rheum.*, 2019, **49**, 337–350.
6 S. L. Kolasinski, T. Neogi, M. C. Hochberg, C. Oatis, G. Guyatt, J. Block, L. Callahan, C. Copenhaver, C. Dodge and D. Felson, *Arthritis Rheumatol.*, 2020, **72**, 220–233.
7 The National Institute for Health and Care Excellence (NICE), *Osteoarthritis in over 16s: diagnosis and management*, Report NG226, 2022.
8 Astra Zeneca, *Pharmaceuticals in the Environment Statement*, Cambridge, U.K., 2024.
9 A. G. Novartis, *Pharmaceuticals in the Environment*, Basel, Switzerland, p. 2023.
10 F. Hoffmann-La Roche AG, *Roche Position on Pharmaceuticals in the Environment (PiE)*, Basel, Switzerland, 2011.
11 F. Hoffmann-La Roche AG, *Roche Position on Product Stewardship*, Basel, Switzerland, 2017.
12 M. Cristofoli, J. Hadgraft, M. E. Lane and B. C. Sil, *Int. J. Pharm.*, 2022, **623**, 121906–121906.
13 M. Cristofoli, J. Hadgraft, M. E. Lane and B. C. Sil, *A model binary system for the evaluation of novel ion pair formulations of diclofenac*, RSC Pharmaceutics, 2024.
14 G. Shazly, N. Haq and F. Shakeel, *Pharmazie*, 2014, **69**, 335–339.
15 International Conference on Harmonisation Expert Working Group, ICH Harmonised Tripartite Guideline Geneva, Switzerland, 2005.
16 A. F. Barton, *CRC Handbook of Solubility Parameters and Other Cohesion Parameters*, CRC Press, 1991.
17 M. J. Louwerse, A. Maldonado, S. Rousseau, C. Moreau-Masselon, B. Roux and G. Rothenberg, *ChemPhysChem*, 2017, **18**, 2999–3006.
18 S. Alshehri and F. Shakeel, *J. Mol. Liq.*, 2020, **307**, 112970.
19 J. Barra, M.-A. Peña and P. Bustamante, *Eur. J. Pharm. Sci.*, 2000, **10**, 153–161.
20 T. Higuchi, *J. Soc. Cosmet. Chem.*, 1960, **11**, 85–97.
21 L. K. Golightly, S. S. Smolinske, M. L. Bennett, E. W. Sutherland and B. H. Rumack, *Med. Toxicol. Adverse Drug Exper.*, 1988, **3**, 128–165.
22 Z. Liron and S. Cohen, *J. Pharm. Sci.*, 1984, **73**, 538–542.
23 Scientific Committee on Consumer Safety (SCCS), *Opinion on diethylene glycol monoethyl ether (DEGEE)*, 2013, pp. 15–16.
24 T. Haque, K. M. Rahman, D. E. Thurston, J. Hadgraft and M. E. Lane, *Eur. J. Pharm. Sci.*, 2017, **104**, 188–195.
25 F. Iliopoulos, B. C. Sil, A. M. Al Hossain, D. J. Moore, R. A. Lucas and M. E. Lane, *Int. J. Pharm.*, 2020, **579**, 119137.
26 A. M. A. Hossain, B. C. Sil, F. Iliopoulos, R. Lever, J. Hadgraft and M. E. Lane, *Pharmaceutics*, 2019, **11**, 548.
27 C.-P. Kung, Y. Zhang, B. C. Sil, J. Hadgraft, M. E. Lane, B. Patel and R. McCulloch, *Int. J. Pharm.*, 2020, **586**, 119538.
28 T. Haque, K. M. Rahman, D. E. Thurston, J. Hadgraft and M. E. Lane, *Eur. J. Pharm. Sci.*, 2018, **121**, 59–64.
29 National Center for Biotechnology Information, PubChem Compound Summary for, Dipropylene glycol. **https://pubchem.ncbi.nlm.nih.gov/compound/Dipropylene-glycol**. Accessed Jan. 13, 2025.
30 National Center for Biotechnology Information, PubChem Compound Summary for CID 8146, Diethylene Glycol Monoethyl Ether, PubChem, **https://pubchem.ncbi.nlm.nih.gov/compound/Diethylene-Glycol-Monoethyl-Ether**. Accessed 13 January, 2025.
31 F. Shakeel, M. Kazi, F. K. Alanazi and P. Alam, *Molecules*, 2021, **26**, 7052.
32 F. Shakeel, N. Haq and S. Alshehri, *Molecules*, 2020, **25**, 2743.
33 F. Shakeel, F. K. Alanazi, I. A. Alsarra and N. Haq, *J. Chem. Eng. Data*, 2013, **58**, 3551–3556.
34 W. Bialik, K. Walters, K. Brain and J. Hadgraft, *Int. J. Pharm.*, 1993, **92**, 219–223.
35 SAS Gattefossé, *Transcutol® P for efficient drug solubilization and skin penetration*, 2018.
36 E. Ikada, *J. Phys. Chem.*, 1971, **75**, 1240–1246.
37 D. W. Osborne and J. Musakhanian, *AAPS PharmSciTech*, 2018, **19**, 3512–3533.
38 J. E. Harrison, A. C. Watkinson, D. M. Green, J. Hadgraft and K. Brain, *Pharm. Res.*, 1996, **13**, 542–546.
39 S. Björklund, Q. D. Pham, L. B. Jensen, N. Ø. Knudsen, L. D. Nielsen, K. Ekelund, T. Ruzgas, J. Engblom and E. Sparr, *J. Colloid Interface Sci.*, 2016, **479**, 207–220.
40 J. Hadgraft, *Int. J. Pharm.*, 2001, **224**, 1–18.
41 G. Oliveira, J. Hadgraft and M. Lane, *Int. J. Cosmet. Sci.*, 2012, **34**, 536–545.
42 OECD, *Test No. 428: Skin Absorption: In Vitro Method*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, 2004, pp. 1–8, DOI: **10.1787/9789264071087-en**.
43 N. Bjerrum, *Untersuchungen über Ionenassoziation*, AF Høst, 1926.
44 C. A. Kraus, *J. Phys. Chem.*, 1956, **60**, 129–141.
45 S. S. Sastry, N. Sarma, T. Vishwam and V. Murthy, *Indian J. Pure Appl. Phys.*, 2017, **55**, 403–412.
46 R. Sengwa, *J. Mol. Liq.*, 2003, **108**, 47–60.
47 T. E. Daubert, *Design Institute for Physical Property Data (DIPPR)*, 1989.
48 G. B. Kasting, W. R. Francis and G. E. Roberts, *J. Pharm. Sci.*, 1993, **82**, 551–552.
49 C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft and R. H. Guy, *J. Pharm. Sci.*, 2008, **97**, 185–197.
50 R. Watkinson, R. Guy, J. Hadgraft and M. Lane, *Skin Pharmacol. Physiol.*, 2009, **22**, 225–230.
51 D. Mohammed, K. Hirata, J. Hadgraft and M. E. Lane, *Eur. J. Pharm. Sci.*, 2014, **51**, 118–122.
52 B. W. Barry, *J. Controlled Release*, 1987, **6**, 85–97.

53 J. Bouwstra, M. De Vries, G. Gooris, W. Bras, J. Brussee and M. Ponec, *J. Controlled Release*, 1991, **15**, 209–219.
54 I. Brinkmann and C. Müller-Goymann, *Pharmazie*, 2005, **60**, 215–220.
55 S. H. Moghadam, E. Saliaj, S. D. Wettig, C. Dong, M. V. Ivanova, J. T. Huzil and M. Foldvari, *Mol. Pharm.*, 2013, **10**, 2248–2260.
56 V. Carrer, C. Alonso, M. Pont, M. Zanuy, M. Córdoba, S. Espinosa, C. Barba, M. A. Oliver, M. Martí and L. Coderch, *Arch. Dermatol. Res.*, 2020, **312**, 337–352.
57 A. Hoelgaard and B. Møllgaard, *J. Controlled Release*, 1985, **2**, 111–120.
58 W. J. Fasano, W. F. ten Berge, M. I. Banton, M. Heneweer and N. P. Moore, *Toxicol. In Vitro*, 2011, **25**, 1664–1670.
59 X. Guo, Z. Guo, H. Wei, H. Yang, Y. He, S. Xie, G. Wu, H. Zhong, L. Li and Q. Zhao, *Laser Phys.*, 2010, **20**, 1849–1855.
60 D. Mohammed, P. Matts, J. Hadgraft and M. Lane, *Pharm. Res.*, 2014, **31**, 394–400.
61 L. Trottet, C. Merly, M. Mirza, J. Hadgraft and A. Davis, *Int. J. Pharm.*, 2004, **274**, 213–219.
62 A. C. Williams and B. W. Barry, *Adv. Drug Delivery Rev.*, 2012, **64**, 128–137.
63 M. E. Lane, *Int. J. Pharm.*, 2013, **447**, 12–21.
64 B. Barry, *J. Controlled Release*, 1991, **15**, 237–248.
65 M. Paz-Alvarez, P. D. Pudney, J. Hadgraft and M. E. Lane, *Int. J. Pharm.*, 2018, **549**, 317–324.
66 I.-M. Schneider, B. Dobner, R. Neubert and W. Wohlrab, *Int. J. Pharm.*, 1996, **145**, 187–196.
67 E. Bonnist, J.-P. Gorce, C. Mackay, R. Pendlington and P. Pudney, *Skin Pharmacol. Physiol.*, 2011, **24**, 274–283.
68 Y. Zhang, C.-P. Kung, F. Iliopoulos, B. C. Sil, J. Hadgraft and M. E. Lane, *Pharmaceutics*, 2021, **13**, 726.
69 Q. Zhang, P. Li and M. S. Roberts, *J. Controlled Release*, 2011, **154**, 50–57.
70 M. A. Yamane, A. C. Williams and B. W. Barry, *J. Pharm. Pharmacol.*, 1995, **47**, 978–989.
71 M. Paz-Alvarez, C. F. Tang, P. D. Pudney and M. E. Lane, *Int. J. Pharm.*, 2024, **653**, 123886.