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Research Paper

# Dynamic in-situ eutectic formation for topical drug delivery

Sarah Fiala[a], Marc B. Brown[b,c] and Stuart A. Jones[a]

[a]Institute of Pharmaceutical Science, King's College London, London, [b]School of Pharmacy, University of Hertfordshire, College Lane, Hatfield, Herts and [c]MedPharm Ltd, Guilford, UK

## Abstract

**Objectives** The relationship between the solution-state chemistry of eutectic systems and their transmembrane transport characteristics is difficult to define as these mixtures are sensitive to delivery vehicle-induced penetration enhancement. Through in-situ formation of a molten eutectic mixture using highly evaporative sprays this study aimed to gain an understanding of solution-state thermodynamic and chemical properties of eutectic combinations pertinent to transmembrane transport in the absence of a delivery vehicle.

**Methods** In-situ molten lidocaine–prilocaine eutectics were formed using a hydrofluouroalkane (HFA) propellant. Transport through silicone membranes and human skin in upright Franz diffusion cells was determined using in-house manufactured creams as controls.

**Key findings** The application of the two drugs in an HFA spray produced a molten oil even when the melting point of the drug mixture was above the experimental temperature at the membrane surface. In the absence of vehicle effects, molecule presentation to the membrane interface was most effective using a lidocaine-rich mixture of 0.7% w/w lidocaine:prilocaine – 1985.06 ± 128.87 μg/h/cm$^2$.

**Conclusions** There appeared to be no link between melting point and transmembrane transport of lidocaine:prilocaine from a eutectic mixture. The rate of drug presentation to the membrane interface, which was highest in drug-rich, high-activity molten eutectic mixtures, was the driver for transmembrane transport in the absence of significant barrier interactions.

**Keywords** eutectic; lidocaine; o/w cream; prilocaine; spray

## Introduction

Combining a therapeutic agent with a second chemical to form a eutectic system (a binary system that when mixed exhibits a melting point that is lower than either of the component agents) is one passive enhancement method that has been shown to improve transmembrane transport.[1] EMLA (eutectic mixture of local anaesthetics), a product that claims superior clinical efficacy compared with either drug applied alone, highlights the commercial viability of the eutectic systems, but the manner in which this product enhances topical drug delivery is still not fully understood.[2] The use of complex formulation vehicles with eutectic systems makes it difficult to interpret the basic solution-state behaviour of the active molecules and there is a need to provide a sound theoretical basis with which to derive a better understanding of these interesting pharmaceutical products.

Simply demonstrating that saturating a one-phase administration vehicle with two drugs reduces the comparative permeation of the two agents in a competitive manner has influenced the understanding of eutectic drug delivery systems.[3] Taking account of the competition that exists between diffusing molecules showed that pure eutectic oils delivered up to three-fold more drug compared with matched systems that administered compounds using an aqueous solution.[3] The transmembrane penetration efficiency of pure eutectic oils when applied as physical mixtures was shown to be a result of improved access of the agents to the membrane interface, changes in solubility in the barrier and a superior diffusivity through the membrane.[4] However, these three drivers of transmembrane transport were influenced by adding a third component to the eutectic (e.g. a formulation vehicle);[5] this phenomena has yet to be fully explained.

In theory, many eutectic systems can be directly administered to the skin without the use of a formulation vehicle as they often form an oil at room temperature. However, the inclusion of a therapeutic agent in a delivery vehicle improves two very important characteristics:

**Correspondence:** Stuart A. Jones, Pharmaceutical Science Division, King's College London, 150 Stamford Street, London SE1 9NH, UK.
E-mail: stuart.jones@kcl.ac.uk

cosmetic appeal and retention at the site of application. Applying the pure oil may be highly efficient, but it would lack cosmetic elegance and thus potentially affect patient acceptance. Finding the balance between efficiency and elegance for eutectic systems is a paradox that is not easily solved using traditional formulation approaches.

TEMPE spray (topical eutectic mixture for premature ejaculation), a formulation developed by Plethora Solutions Plc (London, UK, http://www.plethorasolutions.co.uk) delivers the lidocaine and prilocaine eutectic at a 3 : 1 ratio using Tetrafluoroethane (HFA 134a).[6–8] Delivering a eutectic using a highly volatile hydrofluroalkane (HFA) solvent creates a dynamic delivery system (i.e. a topical formulation that would form the eutectic '*in situ*' on the skin). HFA is known to evaporate very rapidly after administration (within 1–2 min) and thus it would deposit a thin film of pure eutectic oil on the skin surface.[9–11] For a eutectic system this would be ideal as removal of the delivery vehicle after application could reverse the negative effects of additional components upon eutectic combinations that have been observed in previous work.[5] Although the TEMPE system is in clinical development, no published data details the fundamental properties of the transport process for the eutectic or justifies the use of a 3 : 1 ratio when the eutectic ratio is known to be 1 : 1. In addition no data is publicly available on how the formation of a eutectic *in situ* influences its solution-state chemistry (for simplicity this term is also used to make reference to the molten-state chemical properties) and transport characteristics.

Thus the aim of this study was to gain an understanding of how eutectic–vehicle interactions influence the transmembrane penetration of molecules delivered using a eutectic system by studying the permeation behaviour of lidocaine and prilocaine when applied as an HFA spray. To do this, the transport of the lidocaine–prilocaine eutectic system was assessed when the system was dosed using the dynamic spray and a traditional oil-in-water (o/w) cream. A series of lidocaine–prilocaine ratios were used to produce oils with different melting points and, more importantly, different capacities to present lidocaine and prilocaine to the surface of the silicone membrane. Initially a homogeneous synthetic silicone membrane was used in this study to investigate the link between the solution-state chemistry of the molecules and transmembrane penetration properties of the test molecules. This experimental design was employed in an attempt to understand the chemical behaviour of the two drug molecules, lidocaine and prilocaine, when presented as a eutectic system to a hydrophobic surface. It allowed comparison of the generated results to those of previous work on dual drug permeation while avoiding the confounding effects of vehicle–skin interactions, a strategy previously shown to be effective with topical anaesthetics.[12–14] In addition, skin penetration studies were performed to specifically assess the effects of EMLA delivery vehicle on the permeability of the skin. However, there was no attempt to correlate the silicone and skin results as it was not the intention to predict in-vivo behaviour from the silicone membrane data. These two pieces of independently generated but interrelated transmembrane penetration data were discussed in parallel but not combined to facilitate a better understanding of how chemicals penetrate such barriers when delivered using a eutectic mixture.

## Materials and Methods

### Materials

Lidocaine was supplied by QueMaCo (Nottingham, UK) and prilocaine by Chemos GmbH (Regenstauf, Germany). Phosphate-buffered saline (PBS, pH 7.3, 0.15 M) tablets were provided by Oxoid Ltd (Basingtoke, UK). Methanol and de-ionised water, both HPLC grade, were purchased from Fisher Scientific (Loughborough, UK). Arlatone 189 was obtained from Uniquema (Emmerich, Germany) and Carbomer 934P from Libraw Pharma (New Delhi, India). Tetrafluoroethane (Solkane 134a) was kindly donated by Solvay Fluor GmbH (Hannover, Germany). Silicone membrane (Folioxane C6) with a thickness of 0.12 mm was obtained from Novatech SA (La Ciotat, France).

### Preparation of sprays

Lidocaine and prilocaine were weighed directly into a 10 ml Purgard canister made of clear glass and safety coated in polypropylene (Adelphi Tubes, Haywards Heath, UK) at the desired weight/weight ratios. The canister was then sealed with a 50 µl metered valve (Bespak Europe Ltd, King's Lynn, UK). HFA 134a was filled into the sealed glass canister using an pressurised filler (Model # 2016; Pamasol Willi Mader AG, Pfäffikon, Switzerland) until the desired weight was obtained (final total drug concentration was in the range of 22–28% w/w). The canisters were shaken gently to ensure complete dissolution of lidocaine and prilocaine in the HFA.

### Preparation of creams

Lidocaine, prilocaine and the surfactant (Arlatone 189) were melted together under gentle heat (ca. 50°C) at the following ratios: 0.6 : 1.4 : 0.76, 0.8 : 1.2 : 0.76, 1 : 1 : 0.76, 1.2 : 0.8 : 0.76 and 1.4 : 0.6 : 0.76 to produce the oil phase. The Carbomer 934P was completely dissolved in water (final concentration 1% w/v) and then the pH was adjusted to 9.0–9.5, which resulted in a thick clear gel. An appropriate amount of the oil phase (final drug concentration 5%) was then mixed together with the gel using a homogeniser (Silverson L4 Series; Silverson, Chesham, UK) at a rate of 5000 rpm for ca. 10 min to obtain o/w creams.

### Silicone penetration studies

Unjacketed, individually-calibrated, upright Franz diffusion cells (MedPharm Ltd, London, UK) with surface areas of approximately 2.2 $cm^2$ and receiver compartment volumes of approximately 9.5 ml were used for the permeation experiments. Donor and receiver chambers were sealed together using parafilm onto a circular section of silicone membrane (used as obtained). Receiver compartments were filled with PBS (0.172 M, pH 7.3) and stirred with small magnetic bars to ensure adequate mixing and maintenance of sink conditions. A minimum of five diffusion cells were used for each experiment. Franz cells were allowed to equilibrate for 30 min before use by immersing the receiver compartment of the cells in a 25°C water bath (Grant instruments, Cambridge, UK). The integrity of the cells was checked after equilibration by inversion, the appearance of any receiver fluid in the apical

chamber at this point led to cell rejection from the study. The experiment was initiated by the application of an infinite dose of the formulations. Either 25–30 shots of the eutectic spray (spreading not required) or 1.5 ml of the cream (applied using a syringe and smoothed with a spatula) was applied to the apical surface of the silicone membrane such that the whole membrane was covered by the formulations. At specified time intervals, 1 ml samples were taken out of the sampling arm of the receiver compartment and immediately replaced by fresh PBS of equal volume and temperature. Samples were stored at room temperature until HPLC analysis was carried out. Cumulative amounts of drug (μg) penetrating the membrane per unit diffusional surface area of silicone membrane ($cm^2$) were corrected for previous sample removal and plotted against time (h). The slope of the linear portion of the plot ($R^2 \geq 0.99$ over at least six points) was defined as steady-state flux ($J_{ss}$). A temperature of 25°C was used for the silicone membrane studies to allow easy comparison across the transport and solubility experiments in chemically stable systems.

### Determination of drug solubility in silicone membrane

The silicone membrane was cut into squares of 1.5 cm, weighed and placed into vials. An accurately weighed amount (ca. 500 mg) of the spray (applied directly from the canister) or the cream (applied using a spatula) was placed in the vial to completely cover the membrane. The vials were agitated for 24 h in a 25°C shaking water bath (Grant instruments, Cambridge, UK) at a rate of ca. 170 rotations per min. The piece of membrane was removed from the vial and wiped thoroughly with tissue paper. The lidocaine and prilocaine in the membrane were extracted by immersing it in 0.1 M HCl solution for 72 h. The membrane was removed and the HCl samples dried in the oven at 60°C for 4 h, reconstituted in PBS and analysed by HPLC. The extraction method was validated and found to be fit for purpose (recovery ≥100% for both drugs).

### Human skin penetration studies

Surgically excised samples of full thickness human skin were obtained directly after abdominoplastic surgery with informed consent. The skin was stored at −30°C until used for permeation studies. Frozen skin was partially thawed before removal of subcutaneous fat by dissection. This skin was then immersed in de-ionised water at 60°C ± 3°C for 60 s while stirring gently. It was laid dermal side down on aluminium foil and the epidermal layer was manually rolled back gently, using a scalpel to dislocate any edges of skin adhered to the dermal layer. The epidermal sheet was floated in de-ionised water, stratum corneum side up, and immediately taken up onto a sheet of filter paper (Whatman International, Maidstone, UK). The resulting sheet was left to dry and then stored flat in aluminium foil at −30°C. Before the permeation study, the skin was thawed and assembled in Franz cells (surface area ca. 0.6 $cm^2$ and receiver compartment volume ca. 2.2 ml) as described previously. The cells were placed in a 37°C water bath and the permeation study was initiated by applying infinite doses (matched 25 mg total drug load) of the formulations to the apical surface of the skin. Samples were taken from the receiver fluid 1 h after application of the dosage form and at suitable time points over 24 h, the fluid removed was replaced and the lidocaine and prilocaine content passing through the skin determined by HPLC. Note that an infinite dosing protocol was applied for these studies. The systems compared were of different drug concentrations and two types of studies were conducted: one series where the formulations were matched in terms of the mass of drug applied and a second where the systems were matched in terms of the applied mass of formulation (120 mg of total formulation). In theory, if the conditions of the experiment met that of a standard infinite dosing regimen the two sets of studies should give identical trends; they did and hence only the drug matched data is shown.

### HPLC analysis

An LC pump with autosampler (Hewett-Packard series 1050, Agilent Technologies UK Ltd., Wokingham, UK) connected to a UV absorbance detector (HP series 1050), was used for the quantitative determination of lidocaine and prilocaine. This system was connected to a computer with Chromeleon software (Dionex Corp., Sunnyvale, CA, USA), which was used to record and interpret the analytical data. A Gemini C18 (5 μm, 250 × 4.6 mm) column (Phenomenex Ltd., Macclesfield, UK) was used with a 70 : 30 methanol : water mobile phase at pH 10.0 and a flow rate of 1 ml/min. Volumes of 10 μl were injected on the column and the drugs were analysed at a wavelength of 210 nm. The column temperature was maintained at 50°C using a thermostat oven (Jones Chromatography, Tir-y-Berth, UK). The method was previously shown to be fit for purpose in terms of precision (<3%), accuracy (>99%), linearity ($r^2$ >0.999) and sensitivity (the limits of detection, 7.10 μg/ml; 4.45 μg/ml and the limits of quantification, 23.68 μg/ml; 14.82 μg/ml, for prilocaine and lidocaine, respectively).[3]

### Statistical analysis

Statistical evaluation was carried out using a statistical package for social sciences software (SPSS version 15.0; SPSS inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to check the normality of data. A *t*-test was used to compare the steady-state fluxes and drug solubility. However, analysis of more than two groups of data was carried out using one-way analysis of variance and post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. In all cases, $P \leq 0.05$ denoted a statistically significant difference. All values were expressed as mean ± standard deviation. The number of replicates was five in permeation studies and three in membrane solubility studies.

## Results and Discussion

### Eutectic oils

The passive transport of molecules across a confluent membrane is known to be heavily dependent upon the diffusion gradient set up across the membrane.[15] There exists a debate in the literature as to whether the actual drug concentration or the thermodynamic activity of the molecules (i.e. their degree of saturation in the vehicle) should be controlled when both

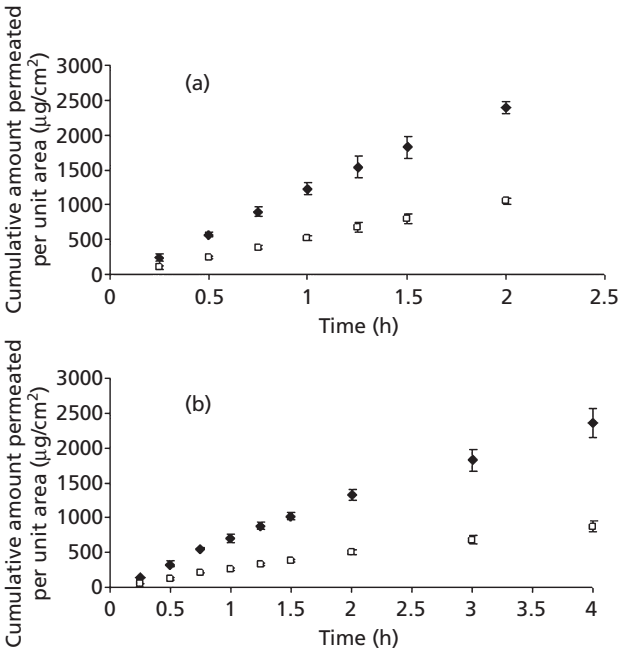


**Figure 1** The permeation profiles of prilocaine (◆) and lidocaine (□) through silicone membrane when applied as a hydroflouroalkane (HFA) spray formulation (a) or an oil-in-water cream (b). In both cases the prilocaine/lidocaine ratio was 0.7 : 0.3. Each point represents the mean ± 1 standard deviation, $n = 5$.

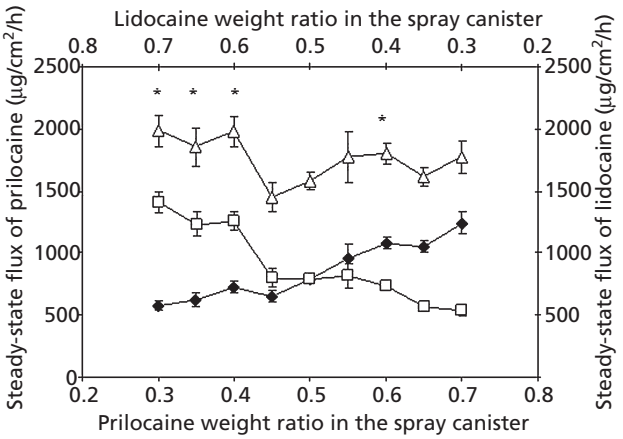


**Figure 2** Relationship between the steady-state flux of lidocaine and prilocaine and their weight ratio when applied as a hydroflouroalkane (HFA) spray: prilocaine (◆), lidocaine (□) and total flux (Δ). Each point represents the mean ± 1 standard deviation, $n = 5$. $^*P < 0.05$, total steady-state flux compared with the 1 : 1 eutectic ratio.

indices are available for manipulation in experiments, but this is dictated by the experimental design and intended outcomes of the investigation. Higuchi's seminal work showed that penetration rate was proportional to thermodynamic activity and independent of drug concentration. The thermodynamic activity-transport relationship in silicone membranes for lidocaine and prilocaine has previously been established.[5,16] Hence, the comparative transport studies reported herein were designed on the basis that when the transport was not limited by dose depletion in the donor system or drug clearance rate at the underside of the membrane (i.e. the maintenance of sink conditions) it was the thermodynamic activity that was the driving force of the passive process. Experimentally it was shown that the permeation profiles of both prilocaine and lidocaine through silicone membrane when applied as a HFA spray were linear ($R^2 \geq 0.99$) over the experiment duration of 2 h. Sink conditions in the receiver fluid were maintained for the first 1.5 h (i.e. the concentration of both prilocaine and lidocaine did not exceed 10% of their maximum solubility in the receiver fluid during this time, <4.7% for prilocaine and <9.8% for lidocaine, respectively, Figure 1) and steady-state flux of the two agents at their eutectic ratio (1 : 1) was not effected by oil concentration in the HFA spray; the drug transport from 44% w/w and 72% w/w oil loadings at 1 : 1 ratio were not to be significantly different, $P > 0.05$, *t*-test (data not represented graphically, 790.7 ± 92.3 μg/cm² per hour and 790.3 ± 34.8 μg/cm² per hour, respectively, for prilocaine; 804.3 ± 113.5 μg/cm² per hour and 792.7 ± 36.3 μg/cm² per hour, respectively, for lidocaine). This series of results demonstrated that the infinite dosing regime was generating steady-state flux independently of the total oil loading on the membrane surface and the criteria required to make the theoretical assumption that thermodynamic activity was the primary driving force were being met in the experimental set up (i.e. free diffusion was not being hindered by drug depletion on the donor side of the membrane or saturation on the receiver side). It is important to note that the transmembrane transport rate values obtained for the prilocaine and lidocaine HFA spray at the 1 : 1 ratio were not significantly different ($P > 0.05$, analysis of variance) from the steady-state fluxes of such drugs previously reported when applied as pure oil at the same ratio (the oil previously generated a rate of 712.05 ± 39.39 μg/cm²/h for prilocaine and 762.70 ± 44.63 μg/cm²/h for lidocaine, respectively).[5] This supported the assumption that the HFA solvent had evaporated within the first 1–2 min, which has also been reported previously.[9,11]

Previous work has shown that the transmembrane penetration rate of the eutectic drugs, prilocaine and lidoacine was competitive, with each agent being transported at a rate that was proportional to its relative thermodynamic activity in the applied vehicle.[3] When formulated as an HFA spray the transport rate across the silicone membrane was also shown to be dependent on the ratio at which they were applied to the apical surface of the membrane, which again demonstrated that the transport was competitive (Figure 2). In previous work when an aqueous solution was used to apply the two agents their transmembrane penetration rate was retarded compared with the pure molten oils. In contrast, incorporating lidocaine-rich or prilocaine-rich mixtures (≥0.6 w/w) in an HFA spray in this study, increased total permeation compared with the molten oils.[5] At several ratios of lidocaine to prilocaine the total drug penetration rate was significantly higher than the eutectic 1 : 1 ratio when administered using the HFA spray. The melting point of pure lidocaine and prilocaine has previously been determined as 67°C and 37°C, respectively, and the 1 : 1 mixture eutectic temperature has been recorded as 18 ± 1°C.[17] Using the melting point data from this previous work it would be theoretically expected that recrystallisation be initiated post spray administration

(i.e. after 1–2 min), when the HFA had evaporated, if the spray was rich in either lidocaine or prilocaine. The transport study experimental temperature, deliberately set at 25°C to ensure stability of the chemicals and methodological equivalence, was below that of the melting temperature of the mixtures and this should have driven liquid to solid transition, but this was not the case. For example, the HFA spray with a prilocaine-:lidocaine ratio of 0.4 : 0.6 produced a single liquid phase free from crystals after actuation (examined microscopically) even though the predicted melting point for this system was ca. 28°C.[17] It was therefore concluded that forming the eutectic of lidocaine and prilocaine dynamically, post dosing, on the surface of the silicone membrane using the HFA propellant formed an intimate pure mixture that was in a transient state of high activity. The study by Brodin *et al*.[17] supports this hypothesis as it showed that the kinetics of eutectic recrystallisation were slow; it took several weeks for the two agents to reach a state of equilibrium that allowed an accurate phase diagram to be constructed. This hypothesis can be used to explain the differences between the HFA spray, which formed the eutectic at the membrane interface, and the pure molten mixtures (reported in previous work), which were formed as a stock solution several weeks before experimentation. The generation of this transient high-energy state was an inherent characteristic of the HFA spray formulation. Across the series of prilocaine/lidocaine ratios the formation kinetics were dependent on the rapid evaporation of the HFA solvent, which was identical in each system. The preparation conditions for the comparator creams were consistent with the molten oil systems. As the kinetics of equilibrium were slow it was thought that the transport rate measured for the agents using the *in vitro* Franz cells was a good representation of the state of equilibrium of the eutectic directly after presentation to the membrane and this was unlikely to significantly change over the time-course of the experiments (i.e. over a period of several hours).

## Lidocaine and prilocaine creams

The steady-state flux of prilocaine and lidocaine when included in the o/w cream does not simply correspond to the transmembrane transport data previously generated for the molten oils and this in part may be a consequence of the composition of these preparations. The o/w cream, in order to allow comparison with EMLA, also comprised Arlatone, which is polyoxyethylene (PEG25) hydrogenated castor oil, as a co-solubiliser, thickner and non-ionic surfactant, to help form the two-phase system. Such excipients are known to interact with hydrophobic barriers to enhance drug absorption and thus the presence of this agent and its potential to influence the membrane fluidity was considered in all direct experimental comparisons with the HFA spray.[18,19]

The presence of the Arlatone and the aqueous vehicle did not change the shape of the lidocaine and prilocaine transmembrane penetration profiles when compared with the HFA sprays; they remained linear up to 1.5 h ($R^2 \geq 0.99$, Figure 1). The plateau after 1.5 h was due to dose depletion, an effect that correlated with previous work by Nyqvist-Mayer *et al*.[20] Sink conditions in the receiver fluid were maintained throughout the 4-h experiment as prilocaine and lidocaine concentrations did not exceed 10% of the saturated solubility (<0.39 mg/ml (5.4%) and 0.38 mg/ml (9.3%), respectively).

It was assumed that the external aqueous phase of the o/w emulsion was saturated with drug and that the rate of partitioning from the oil into the external phase would govern the replenishment of the drug that was available for permeation (Figure 3). Comparing the transmembrane penetration rates of the lidocaine and prilocaine from the creams obtained in this work with those obtained from previous work in an identical

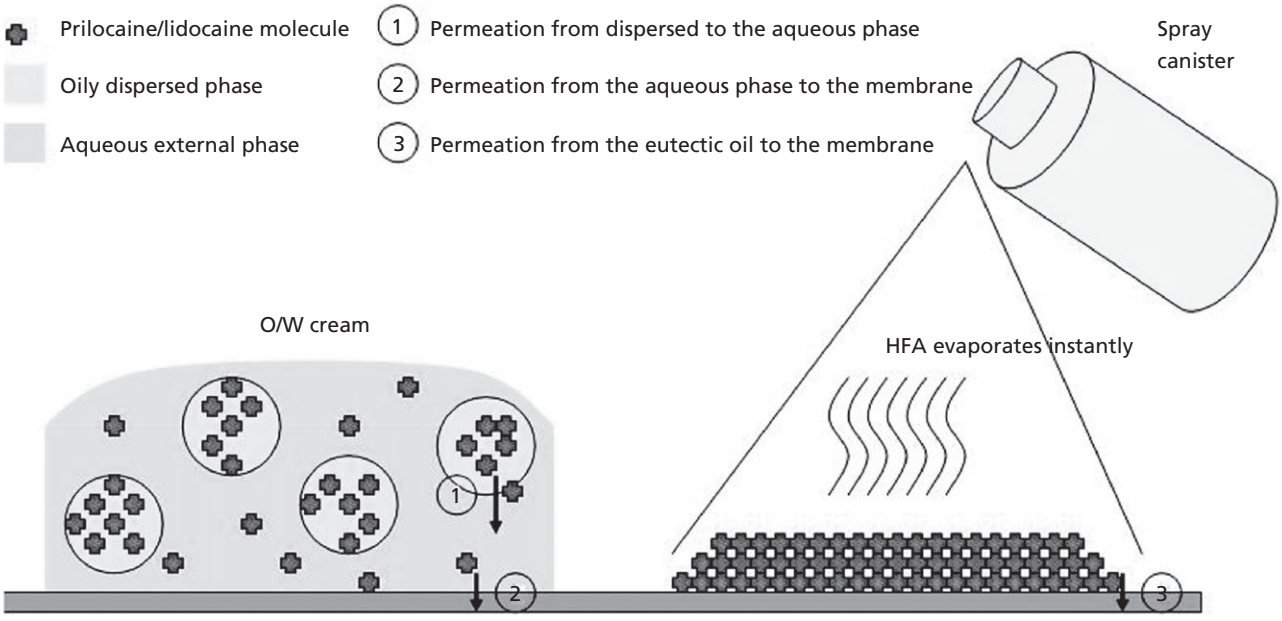


**Figure 3** Diagram showing the different presentation of the local anaesthetics lidocaine and prilocaine to the surface of a membrane after application from either a hydroflouroalkane (HFA) spray or an oil-in-water cream.

experimental set up using a simple aqueous administration vehicle, it is evident that the drug ratio/transmembrane penetration rate trends were similar, but the creams show an enhanced penetration of ca. 1.5–2.0 fold.[3] This enhancement was assumed to be due to the effects of Arlatone on the membrane because in both systems the drug was passing through an aqueous solution in an un-ionised state to reach the membrane (note this was controlled, see experimental section). It is interesting to note that the total steady-state flux of prilocaine and lidocaine from eutectic emulsions has previously been suggested to be dependent on the concentration of the oil in the eutectic cream, but the penetration enhancer, Arlatone, was increased with increasing oil concentration across the formulations in this previous work.[20] The fact that influence of Arlatone was not investigated renders the correlation between oil loading and penetration questionable.[20] In the current study Arlatone was controlled and constant across the creams containing the differing ratios of lidocaine and prilocaine at a single oil loading. This experimental design isolates the effects of Arlatone when the data is referenced to transport from simple saturated solutions performed in previous work[3] and indicates that penetration enhancement observed with the creams was linked to Arlatone's effects.[21,22] Again, even in the presence of Arlatone, there was a relationship between the steady-state flux and the ratio of the two drugs in the dispersed phase of the cream; this relationship showed a greater similarity to that of the aqueous system compared with the pure oils (Figure 4).[3,5] This re-enforces that transport competition, which was observed in all test systems independently of mode of drug application, is a characteristic of the eutectic systems (Figure 4). Unlike the HFA spray, there was no significant difference in the total steady-state flux through silicone membrane when lidocaine and prilocaine were included in the o/w creams at different drug ratios (Figure 4, $P > 0.05$, analysis of variance). This suggested that the thermodynamic activity of prilocaine and lidocaine together had reached equilibrium in the external phase of the o/w creams, an effect that has previously been shown to be achieved much more rapidly in solution than in the oils.[5]

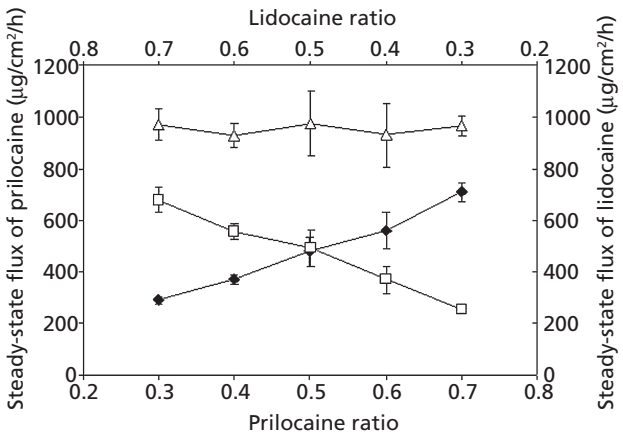


**Figure 4** Relationship between the steady-state flux of lidocaine and prilocaine and their weight ratio in the dispersed phase when applied as an oil-in-water cream: prilocaine (◆), lidocaine (□) and total flux (Δ). Each point represents the mean ± 1 standard deviation, $n = 5$.

The transmembrane penetration rate of lidocaine and prilocaine when applied using the HFA spray was ca. two-fold higher compared with the o/w creams (Table 1). It should be noted that the HFA sprays, unlike the creams, did not contain a potential penetration enhancer and so the true chemical potential difference in activity across the two systems could be greater than the two-fold enhancement observed. In addition, it should be noted that there was a very small proportion of drug that was ionised in the creams. At pH 9.0–9.5, the level of ionisation of lidocaine ranges from 3.1–9.3% and that of prilocaine from 2.4% to 7.2%. Although a relationship between pH and the steady-state flux of lidocaine has been reported, the level of ionisation in the cream was constant and relatively low which means that it should not be a major influence in the transmembrane transport.[23–25] The degree in transmembrane transport differences across the two types of administration systems, cream and HFA spray, differed to some extent with each prilocaine/lidocaine ratio and was lowest at the 1 : 1 eutectic ratio, but the effect, although statistically significant ($P \leq 0.05$, analysis of variance), as a proportion of total enhancement was relatively small.

## Barrier affinity

One of the main determinants of transmembrane penetration is the activity coefficient in the barrier ($C_{s,b}$) as it drives the partitioning process of the drug from the formulation into the membrane (Equation 1):[26]

$$J = (D_b C_{s,b/h})(C_v / C_{s,v}) \quad (1)$$

where $J$ is the flux, $D_b$ is the diffusion coefficient of the drug in the barrier, $C_{s,b}$ is its solubility in the barrier, $h$ is the

**Table 1** Permeation and silicone membrane solubility enhancement ratios of the hydroflouroalkane (HFA) spray formulation over the oil-in-water cream for prilocaine and lidocaine

| Prilocaine/lidocaine ratio (w/w) | Prilocaine | | Lidocaine | |
|---|---|---|---|---|
| | $ER_{permeation}$ | $ER_{solubility}$ | $ER_{permeation}$ | $ER_{solubility}$ |
| 0.3/0.7 | 1.98 ± 0.15 | 1.30 ± 0.02 | 2.08 ± 0.18 | 0.96 ± 0.01 |
| 0.4/0.6 | 1.97 ± 0.15 | 1.13 ± 0.01 | 2.26 ± 0.16 | 1.02 ± 0.02 |
| 0.5/0.5 | 1.63 ± 0.24 | 1.11 ± 0.01 | 1.65 ± 0.32 | 1.02 ± 0.01 |
| 0.6/0.4 | 1.95 ± 0.27 | 1.06 ± 0.00 | 2.00 ± 0.30 | 1.00 ± 0.01 |
| 0.7/0.3 | 1.75 ± 0.14 | 0.98 ± 0.08 | 2.10 ± 0.15 | 0.93 ± 0.08 |

$ER_{permeation}$, permeation enhancement ratio; $ER_{solubility}$, enhancement ratio. Each value represents the mean ± 1 standard deviation, $n = 5$ for permeation and $n = 3$ for solubility in silicone membrane.

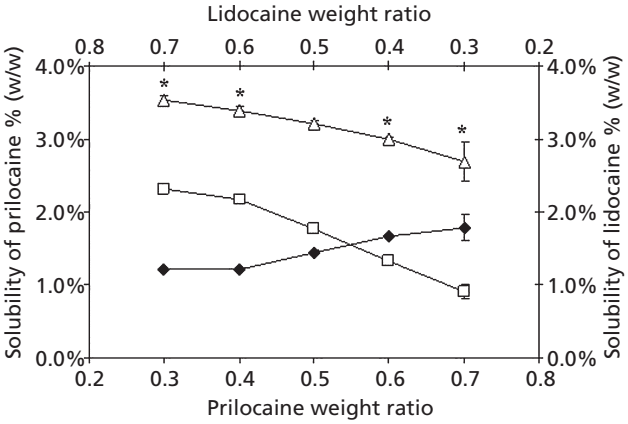


**Figure 5** Relationship between the solubility of prilocaine and lidocaine in silicone membrane and their weight ratio when applied as a hydroflouroalkane (HFA) spray formulation: prilocaine (◆), lidocaine (□) and total solubility (Δ). Each point represents the mean ± 1 standard deviation, $n = 3$. $^*P < 0.05$, total drug solubility in silicone membrane compared with the 1 : 1 eutectic ratio.

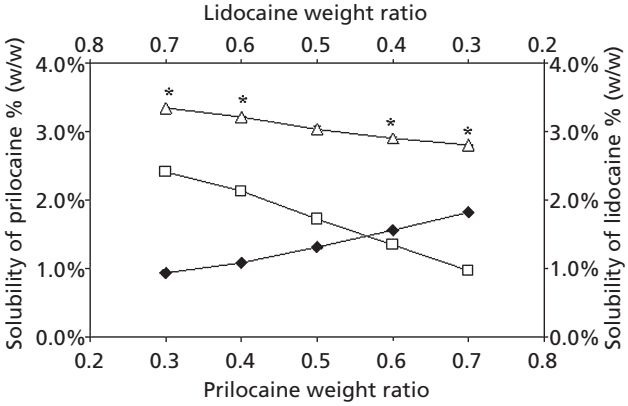


**Figure 6** Relationship between solubility of prilocaine and lidocaine in silicone membrane and their weight ratio of in the dispersed phase when applied as an oil-in-water cream: prilocaine (◆), lidocaine (□) and total solubility (Δ). Each point represents the mean ± 1 standard deviation, $n = 3$. $^*P < 0.05$, total drug solubility in silicone membrane compared with the 1 : 1 eutectic ratio.

diffusion path length across the barrier, $C_v$ is the actual concentration in the vehicle and $C_{s,v}$ is the solubility of the drug in the vehicle, hence this ratio is the thermodynamic activity component of this relationship. Previous studies that attempted to understand the enhancement of drug eutectic combinations suggested that an increased solubility of the drug in the barrier due to the lower melting point of the combined agents, was the major reason for the observed permeation enhancement over the individual agents.[27–29] These studies, however, did not determine the solubility in the barrier or the partitioning behaviour of the eutectic components. A study by Kaplun-Frischoff and Touitou[30] showed that the testosterone partition coefficient in an n-octanol/water system was not affected by eutectic formation with menthol despite the latter decreasing the melting point and enhancing the permeation of testosterone through nude mouse skin. A more recent study on lidocaine–prilocaine eutectic mixtures demonstrated an increase in silicone membrane solubility when the drugs were applied as a pure eutectic mixture as opposed to an aqueous binary saturated solution, but this was attributed to the absence of vehicle and not the decrease in melting point.[3] In the current work, the solubility of lidocaine and prilocaine in silicone membrane when applied as an HFA spray or an o/w cream was measured at different ratios (Figures 5 and 6). Solubility trends were similar to those previously recorded for pure physical mixtures (i.e. the solubility of each drug in silicone membrane was dependent on its ratio in the formulation).[5] The total solubility of the two agents increased significantly as the lidocaine proportion in the drug mixture increased, which can be explained by more lipophilic lidocaine (log P –2.44, ChemIDplus database) entering the membrane and buffering the extreme lipophilicty of silicone to encourage prilocaine (log P –2.11, ChemIDplus database) entry. The preferential affinity of lidocaine for the silicone membrane was demonstrated practically by the fact that lidocaine solubility at the 1 : 1 ratio was significantly higher than that of prilocaine when applied as either an HFA spray or a cream (1.76 ± 0.02% w/w vs 1.45 ± 0.02% w/w for the HFA spray, respectively, $P \leq 0.05$, *t*-test; and 1.73 ± 0.02% w/w vs 1.31 ± 0.02% for the o/w cream, respectively, $P \leq 0.05$, *t*-test). A membrane partition coefficient cannot be experimentally determined for the pure oil systems as the high concentration of the applied system, which cannot be altered in the absence of a vehicle, does not allow free diffusion in and out of the membrane. The two-fold increase in the transmembrane penetration of both prilocaine and lidocaine when the agents were delivered using the HFA spray compared with the o/w cream, even when the effects of the penetration enhancer are considered, cannot be explained by the membrane solubility data which did not show any enhancement for lidocaine and relatively minor enhancement for prilocaine when the agents were presented to the membrane as the HFA spray (Figures 5 and 6). The inability to explain the increase in steady-state transmembrane flux achieved by the HFA sprays through changes in drug partitioning suggest the means by which the application vehicle presents the penetrating molecules, outlined previously, is the main contributory factor. This is a similar conclusion to that reached in the previous study, which compared transmembrane penetration of lidocaine and prilocaine in a simple aqueous solvent with that of the pure physical molten mixture.[3]

## Human skin penetration

Both the data generated herein and from previous studies suggest that the ability to present the penetrating species at a high density at the membrane interface is more important than the increase in the membrane solubility in the barrier when a silicone membrane is used.[3,5] The silicone barrier was used herein to understand the fundamental principles of the solution-state chemistry that was occurring in the application vehicle. These principles in theory can be applied to other systems even if the barrier shows different permeability or physicochemical properties as they have been generated using a homogeneous confluent barrier in a controlled manner. When considering other barriers, what does change is the relative contribution of the penetration enhancer capability of

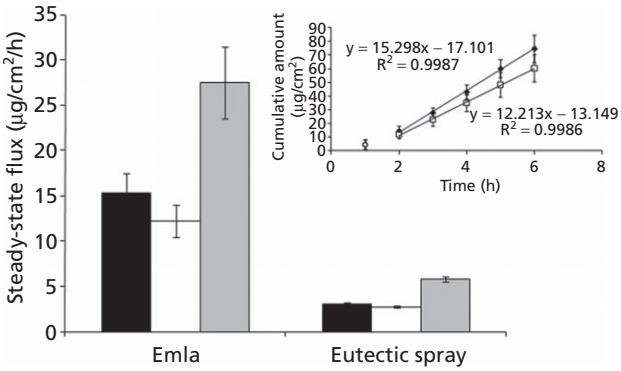


**Figure 7** Steady-state flux of prilocaine (black), lidocaine (white) and the two combined (grey) across human epidermal sheet. Inset is an example permeation profile for EMLA. Each point represents the mean ± 1 standard deviation, $n = 6$.

the cream vehicle. The comparison of the transmembrane penetration capability of the HFA spray and the cream using human epidermal skin highlights this point (Figure 7). The total drug penetration from EMLA cream is ca. five times that of the HFA spray when tested in human skin. Given that the solution-state chemistry has been thoroughly explained for the test systems in this paper (i.e. the drug presentation to the skin surface is known to be greater for the HFA spray), the higher penetration of lidocaine and prilocaine from the ELMA cream can be assigned to the effects of the penetration enhancer on the barrier properties of the skin. The discrepancy in silicone and epidermal skin penetration is not unusual and does not invalidate the data presented herein. Rather it shows the importance of the penetration enhancer system in the ELMA cream and confirms that this is a confounding issue in the work of Nyqvist-Mayer *et al.*,[20] which used a different ratio of surfactant in the preparations. The use of penetration enhancers in the studies that correlated melting point with the transmembrane penetration enhancement could have also confounded this direct link, which was not shown in the current study.[30]

## Conclusions

HFA spray presents lidocaine and prilocaine to the surface of a confluent barrier efficiently through the deposition of a highly activity molten oil. As the HFA propellant evaporates rapidly the two drugs melt *in situ* on the surface of the topical membrane, avoiding the requirement for a formulation vehicle, and generate a dynamically formed eutectic. The maximum total steady-state transmembrane flux of both local anaesthetics was achieved when lidocaine-rich drug mixtures were employed, which could explain the 3 : 1 ratio in the TEMPE spray. When the oil was at this ratio it did not have time to equilibrate and this lack of equilibrium appeared to drive more of the drugs across the silicone membrane. The melting point of a eutectic was shown not to be related to the transmembrane penetration rate of a eutectic system and the partition of the drug into the barrier was not the major contributory factor to penetration through silicone. When tested in human skin the penetration enhancer, included as an emulsifier in EMLA cream, appeared to dominate the drug penetration and made it difficult to gain a mechanistic understanding of how eutectic systems influenced transmembrane penetration using such a biological barrier.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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