International Journal of Pharmaceutics 393 (2010) 68–73

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A fundamental investigation into the effects of eutectic formation on transmembrane transport

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ARTICLE INFO

Article history:
Received 11 December 2009
Received in revised form 1 April 2010
Accepted 1 April 2010
Available online 9 April 2010

Keywords:
Eutectic
Permeation
Silicone membrane
Lidocaine
Prilocaine

ABSTRACT

Eutectic systems enhance the permeation of therapeutic agents across biological barriers, but the mechanism by which this occurs has not previously been elucidated. Using human skin it has proven difficult to isolate the fundamental effects of eutectic formation on molecule diffusion and partition from those that arise as a consequence of the simultaneous application of two agents. The aim of this work was to employ a model hydrophobic membrane to understand the fundamental permeation characteristics of two agents when applied as a eutectic mixture. Lidocaine and prilocaine were selected as model agents and infinite-dose permeation studies were carried out using pre-calibrated Franz diffusion cells with two thicknesses of silicone membrane. Membrane solubility was determined by HCl solution extraction and the membrane diffusion coefficients were calculated from the permeation lag-times. The maximum permeation enhancement was achieved using a eutectic mixture at a 0.7:0.3 prilocaine/lidocaine ratio. A higher solubility of both agents in silicone membrane, enhanced diffusivity of prilocaine and superior release of both drugs, all contributed to produce enhanced permeation from the eutectic mixtures. Deconvolution of the transmembrane transport process suggests that the eutectic enhancement phenomena is a consequence of more favorable permeation characteristics of the two molecules in the absence of a formulation vehicle which competes in the transport process.

1. Introduction

Eutectic systems are typically two-component mixtures that exhibit dramatically different physicochemical properties when compared to the constituent agents. These unique properties arise as a result of only moderate molecular interaction such that when the eutectic mixtures are analysed the discrete characteristics of the molecules are largely retained. For example, combining lidocaine and prilocaine in a binary eutectic system influences the melting point (mpt) of the mixture such that it is dramatically lower than that of either of the individual compounds; however simple infrared spectroscopy analysis shows no change in the molecular orientation of the molecules. The lidocaine:prilocaine eutectic generates a mpt which is below the skin surface temperature, i.e. 32 °C, and this molten liquid has been shown to penetrate the skin more rapidly than would be predicted when applying the agents alone (Woolfson et al., 2000; Yuan and Capomacchia, 2005).

Several studies have attempted to define the relationship between the mpt of a drug and its flux through the skin using mathematical models to interpret experimental data (Kasting et al., 1987; Touitou et al., 1994). According to the ideal solution theory, depressing the mpt of a material increases its solubility in a given solvent including skin lipids (Benson, 2005). However, despite theoretical approaches suggesting that enhanced eutectic penetration through the *SC* is a result of the increase in solubility in the barrier, the multiple effects that occur when applying compounds to a biological barrier such as the skin have made it extremely difficult to generate definitive data to test this hypothesis.

Eutectic mixtures have been employed to deliver agents topically since 1889 when Bonain's liquid was originally prepared. This original mixture consisted of phenol, cocaine and menthol in equivalent proportions (Bonain, 1907; Nortier et al., 1995). It was proven to be highly effective when applied to mucosal surfaces but a clear mechanistic understanding of permeation was not described (Carrasco et al., 1993). More recently similar effects have been shown when ibuprofen was combined with terpenes (Stott et al., 1998), menthol (Yong et al., 2003) and methyl nicotinate (Woolfson et al., 2000) to form a eutectic. This resulted in an increase in its permeation through the skin. The permeation of propranolol (Stott et al., 2001) and lidocaine (Kang et al., 2000) has also been shown to increase when combined with fatty acids and menthol, respectively. Many of these previous studies have referred to

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doi:10.1016/j.ijpharm.2010.04.001

the mpt theory and/or increase membrane solubility to explain the mechanism by which eutectics enhance drug permeation through the skin (Stott et al., 1998, 2001; Kang et al., 2000; Woolfson et al., 2000), but there are several other important factors that should also be considered such as the effects of the excipients on the skin and the dual drug permeation process.

One reason why the mechanism by which eutectic mixtures facilitate effective drug delivery into the skin is not fully understood is because the formulations used in previous work could be altering the barrier properties of the skin. EMLA® cream emulsifies the eutectic in an aqueous vehicle using a surfactant, but the effect of this surfactant or the aqueous external phase on the skin's barrier properties has not been investigated. TEMPE spray (topical eutectic mixture for premature ejaculation), which claims to be superior in a number of characteristics compared to EMLA® cream, administers a pure lidocaine–prilocaine mixture to the skin without the aqueous vehicle, but it uses highly volatile solvents that also could alter skin integrity (Henry et al., 2008). In addition, the majority of published studies designed to assess the percutaneous penetration of eutectic combinations have compared the permeation of binary mixtures to preparations containing a single agent. For example, the clinical efficacy of the commercial lidocaine–prilocaine eutectic cream (EMLA) has been previously compared to individual lidocaine and prilocaine creams (Juhlin et al., 1980). The permeation of lidocaine from lidocaine–menthol eutectic mixtures through shed-snake skin has been compared to a lidocaine saturated solution in phosphate buffer (Kang et al., 2000). Similarly, the rate of ibuprofen-terpene eutectic permeation through human epidermis was compared to an ibuprofen saturated solution (Stott et al., 1998). The permeation of one drug can dramatically inhibit the permeation of the second when applying binary drug solutions to topical membranes means that simply saturating a single agent in an application vehicle is not an ideal control when attempting to measure the permeation of agents from eutectic systems (Fiala et al., 2008).

The aim of this work was to understand the fundamental permeation characteristics of two agents from a eutectic mixture and use this information to investigate the mechanism by which eutectics enhance transmembrane drug transport. In order to achieve this aim lidocaine and prilocaine were employed as model therapeutic agents and their permeation through a silicone membrane was studied. Human skin was not used as the barrier as it is known to be a complex heterogeneous membrane that makes the investigation of complex eutectic systems extremely challenging (Santos et al., 2009). An artificial silicone membrane, which was previously found to be a good model for local anaesthetic permeation through the skin, allows the diffusion path length employed in the studies to be controllably manipulated and this can be used to good effect to understand membrane diffusion (Garrett and Chemburk, 1968; Woolfson et al., 1988, 1998). The permeation from pure eutectic mixtures was compared to that from binary saturated solutions in an attempt to determine the effect of the vehicle. It was hypothesised that there could be four main reasons for the enhanced permeation from eutectics: improved drug release, enhanced solubility in the membrane, a reduction in membrane thickness and an enhancement in membrane diffusion. The influence of these parameters on permeation was isolated and investigated.

2. Materials

Lidocaine base was supplied by QueMaCo (Nottingham, UK) and prilocaine base by Chemos GmbH (Regenstauf, Germany). Phosphate buffered saline tablets were supplied by Oxoid Ltd. (Basingtoke, UK). Methanol and deionised water, both HPLC grade, were purchased from Fisher Scientific (Leicestershire, UK). Silicone membrane (PDMS) with thickness of 0.12 mm (Folioxane® C6) was obtained from Novartis Ltd. (Cedex, France) and the 1 mm membrane was purchased from Bioplexus (Ventura, CA, USA).

3. Methods

3.1. Eutectic mixture preparation

Lidocaine and prilocaine were directly combined in glass vials. The two components were mixed using a spatula and a whirlmixer (Fisons, UK) until a visually homogenous liquid or suspension was obtained. The mixtures were allowed to equilibrate at room temperature (21–25 °C) for 24 h prior to use. Lidocaine to prilocaine ratios of 3:7, 4:6, 5:5, 6:4 and 7:3 (w/w) were prepared and their liquid phase compositions tested. The eutectic mixture solid-liquid equilibrium could be predicted from the phase diagram (Brodin et al., 1984), but the exact composition was assessed experimentally to confirm if the theoretical predictions were correct under the experimental conditions used for the transport studies. So as not to assume the outcome of these studies we used a generic nomenclature for the binary eutectic mixtures. To measure the actual liquid lidocaine/prilocaine component of the mixtures they were centrifuged at 12,000 rpm (Biofuge, Heraeus, Germany) and aliquots of the liquid phase were transferred into vials. The samples were diluted and analysed using high performance liquid chromatography (HPLC).

3.2. Franz diffusion cell studies

Unjacketed, individually calibrated, upright Franz diffusion cells (MedPharm Ltd., UK) with surface areas of approximately 2.2 cm^2 and receptor compartment volumes of approximately 9.5 ml were used for the permeation experiments. Donor and receptor chambers were sealed together using parafilm onto a circular section of silicone membrane (used as obtained). Receptor compartments were filled with PBS (pH 7.3, 0.172 M) 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 prior to use by immersing the receptor compartment sections in a 25 °C water bath (Grant instruments, Cambridge, UK). Approximately 500 mg of the test item was applied to the apical surface of the silicone membrane to initiate the permeation study. At specified time intervals over a period of 2 h, 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 plot ($R^2 \geq 0.99$ over at least 6 points) was defined as steady-state flux (J_{ss}). Membrane thickness was measured before and after the permeation experiments using a Venier micrometer (No. 436.1 Series 0–25 mm) purchased from Starrett (Jedburgh, Scotland, UK).

3.3. Silicone membrane solubility

A series of lidocaine–prilocaine eutectic mixtures (preparation method described in Section 3.1) and binary saturated solutions were tested for maximum drug solubility in silicone membrane. The binary saturated solutions were prepared from single lidocaine base or procaine base solutions in phosphate buffer (0.172 M, final pH 9.57 ± 0.09, concentration ranged from 1 to 4 mg ml^{-1}) to which excess of the counter drug was added. The resulting suspensions were stirred for 24 h in order to reach equilibrium and then filtered using 0.2 μm cellulose acetate syringe filters (recovery was > 0.99)

to obtain clear binary solutions; this method was described elsewhere (Fiala et al., 2008). In order to measure the solubility in the membrane, it was cut into squares of 1.5 cm, weighed and placed into vials. A ca. 500 mg aliquot of the pure mixtures or the saturated solutions was placed in the vial containing the membrane. The vials were agitated for 24 h in a 25 °C shaking water bath at a rate of ca. 170 min^{-1}. The piece of membrane was removed from the vial and wiped with a paper towel. The drugs were extracted from the membrane by immersing in 0.1 M HCl solution for 72 h. The membrane was then removed from HCl solution and discarded. The vials containing solution were placed in an oven at 60 °C for 4 h to evaporate the solvent. The residue remaining in the vial after HCl solution evaporation was reconstituted using phosphate buffer and the lidocaine and prilocaine content analysed by HPLC. The drug recovery using this membrane extraction procedure was ca. 100% for both lidocaine and prilocaine.

3.4. Diffusion coefficient determination

As the permeation of lidocaine and prilocaine through silicone membrane is known to be relatively rapid, a thicker membrane (1 mm) was used in order to obtain reproducible permeation lag-times that would allow the calculation of diffusion coefficients. The results obtained in this section were considered in isolation and were not related to the permeation experiments detailed in Section 3.2 due to the different membrane thickness. The donor fluid was either (1) individually saturated lidocaine and prilocaine solutions, (2) a binary saturated solution or (3) a eutectic mixture. Only one ratio of the eutectic mixture and one ratio of the binary saturated solution were tested. The normalised ratios of prilocaine and lidocaine in the phosphate buffer solution (0.436 and 0.564, respectively) were approximately equivalent to those in the liquid phase of the physical mixture (0.425 and 0.575, respectively). The diffusion coefficients (D) were calculated using the lag-time (L) and membrane thickness (h) according to Eq. (1).

$$L = \frac{h^2}{6D} \quad (1)$$

3.5. HPLC analysis

A LC pump with autosampler (Hewett-Packard series 1050) connected to a UV absorbance detector (HP series 1050) were used for the quantitative determination of lidocaine and prilocaine. This system was connected to a computer with Chromeleon software (Dionex, Surrey, UK), which was used to record the analytical data. A Gemini™ C18 (5 μm, 250 mm × 4.6 mm) column (Phenomenex, Cheshire, UK) was used with a 70:30 methanol:water mobile phase (apparent pH 10) and a flow rate of 1 ml min^{-1}. 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, Mid Glamorgan, 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 were 7.10 μg ml^{-1} and 4.45 μg ml^{-1} and the limits of quantification were 23.68 μg ml^{-1} and 14.82 μg ml^{-1}, for prilocaine and lidocaine, respectively) (Fiala et al., 2008).

3.6. Statistical analysis

Statistical evaluation was carried out using a statistical package for social sciences software (SPSS® version 11.0, 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, drug solubility and diffusion coefficients. Enhancement ratios were analysed by one way ANOVA and post hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. In all cases, a statistically significant difference was defined as when $p \leq 0.05$. All values were expressed as mean ± standard deviation. The number of replicates was 5 in permeation studies and 3 in membrane solubility studies.

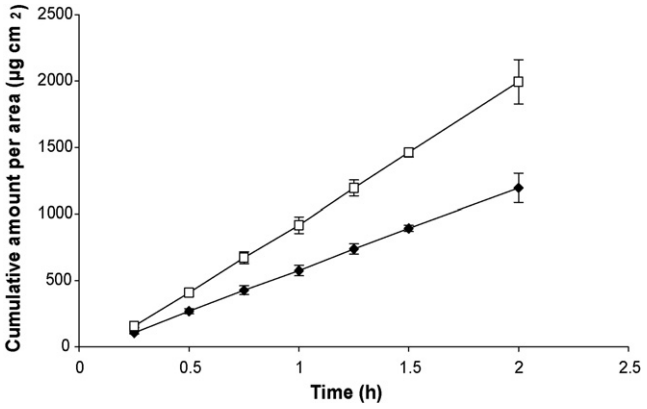

Fig. 1. Permeation profiles of prilocaine (♦) and lidocaine (□) through silicone membrane from a eutectic mixture containing the two drugs at a ratio of 3:7, respectively. Each point represents mean ± standard deviation ($n = 5$).

4. Results and discussion

4.1. Permeation studies

Both lidocaine and prilocaine displayed linear permeation profiles through the 0.12 mm thick silicone membrane over the 2 h experiment time frame ($R^2 \geq 0.99$, Fig. 1). The infinite dosing of the test compounds in the donor compartment prevented dose depletion (only 1.4 ± 0.2%, w/w of the donor drug payload permeated through the membrane in 2 h). In addition, sink conditions were maintained for each experiment; prilocaine and lidocaine concentrations in the receiver fluid did not exceed 10% of the saturated solubility (<0.26 mg ml^{-1} (3.7%) and 0.25 mg ml^{-1} (6.1%), respectively). The steady-state flux of prilocaine and lidocaine from the 1:1 eutectic mixture was found to be 712.05 ± 39.39 μg cm^{-2} h^{-1} and 762.70 ± 44.63 μg cm^{-2} h^{-1}, respectively, which was considerably lower than previously published data. A similar lidocaine–prilocaine eutectic mixture (1:1) demonstrated a steady-state silicone flux of 1554.5 ± 26.2 μg cm^{-2} h^{-1} for prilocaine and 1788.5 ± 30.4 μg cm^{-2} h^{-1} for lidocaine at 32 °C (Nyqvist-Mayer et al., 1986). The differing results could be attributed to the higher temperature in the previously published study (our experiments were conducted at 25 °C) which has a direct effect on passive diffusion of molecules (Williams, 2003). The stokes-Einstein equation (2) relates the diffusion coefficient of a molecule directly to the absolute temperature.

$$D = \mu_p k_B T \quad (2)$$

where D is the diffusion coefficient, μ_p is the mobility of the particle, k_B is Boltzmann's constant and T is the absolute temperature. Increasing temperature increases the kinetic energy and diffusivity of the permeant and thus enhances its permeation.

The two-component phase diagram of the lidocaine–prilocaine system has been previously elucidated using standard thermal techniques and published (Brodin et al., 1984). This previous work showed the mpt of lidocaine and prilocaine to be 67 °C and 37 °C, respectively; the eutectic temperature was 18 ± 1 °C and the eutectic composition was 1:1. Hence, at the experimental temperature employed in the current study (25 °C), the pure prilocaine–lidocaine mixtures were not all expected to be one

Table 1
Permeation of prilocaine and lidocaine through silicone membrane from eutectic mixtures (enhancement ratios were calculated using permeation from binary saturated solutions as a reference). Each point represents mean ± standard deviation ($n = 5$).

Ratio in the mixture (solid and liquid phases) (prilocaine/lidocaine)	Ratio in the liquid phase (prilocaine/lidocaine)	Steady-state flux of prilocaine ($\mu g\,cm^{-2}\,h^{-1}$)	ER	Steady-state flux of lidocaine ($\mu g\,cm^{-2}\,h^{-1}$)	ER
0.3/0.7	0.42/0.58	696.30 ± 45.33	2.27 ± 0.15	1230.41 ± 84.36	4.08 ± 0.28
0.4/0.6	0.48/0.52	627.19 ± 18.67	1.80 ± 0.05	1044.09 ± 28.42	3.84 ± 0.10
0.5/0.5	0.50/0.50[a]	712.05 ± 39.39	1.96 ± 0.11	762.70 ± 44.63	2.92 ± 0.17
0.6/0.4	0.60/0.40[a]	991.21 ± 90.92	2.28 ± 0.21	693.27 ± 57.86	3.31 ± 0.28
0.7/0.3	0.68/0.32	1299.88 ± 163.29	2.62 ± 0.33	550.97 ± 59.88	3.33 ± 0.36

[a] At these ratios, there were no solid particles in the mixture, thus the ratio was assumed to be the same as the initial mixing ratio.

phase systems; solid particles should exist in mixtures with high contents of lidocaine (>0.57) or prilocaine (>0.60) (Brodin et al., 1984). However, when the ratio of the drugs in the liquid phase of the mixtures was determined experimentally, some of the combinations of lidocaine and prilocaine did not show ideal behaviour (Table 1). At prilocaine/lidocaine weight ratios of 0.5/0.5 and 0.6/0.4, there were no solid particles in the mixture which was in agreement with the phase diagram, but when the initial mixing ratio of lidocaine was 0.6, the ratio in the liquid phase was 0.52, which was lower than its saturated solubility according to the phase diagram (0.57). In addition, when the prilocaine ratio in the initial mixture was 0.7, its ratio in the liquid phase was 0.68, i.e. above the level of saturation (0.60). The discrepancies between the results obtained experimentally and those derived from the phase diagram suggest that the pure eutectic mixtures were not at equilibrium when the compositions were tested, i.e. after 24 h. The phase diagram for the two agents determined by Brodin et al., was produced after storage of the mixtures for several weeks at 4–5 °C before examination (Brodin et al., 1984). This indicates that reaching equilibrium between solid and liquid phases in lidocaine–prilocaine mixtures is a slow process.

To avoid any unequivalencies, the individual steady-state fluxes of lidocaine and prilocaine from the eutectic mixtures as well as their total flux were plotted against the actual ratio in the liquid phase as opposed to the initial mixing ratio (Fig. 2). The drug permeation was dependent on its ratio in the applied eutectic; increasing the proportion of an individual agent in the applied mixture increased its steady-state flux. This trend was similar to the results previously reported for the binary lidocaine–prilocaine phosphate buffer solutions hence they imply a similar competition phenomenon was occurring at the membrane surface (Fiala et al., 2008).

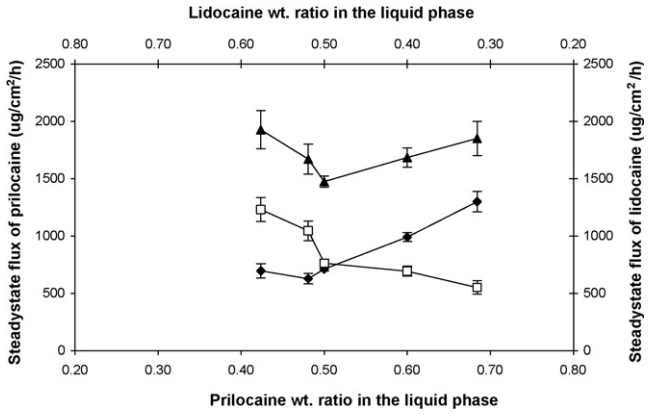

Fig. 2. Relationship between steady-state flux of lidocaine and prilocaine from eutectic mixtures and the weight (wt) ratio in the liquid phase of the mixture: prilocaine (♦), lidocaine (□) and total flux (▲). Each point represents mean ± standard deviation ($n = 5$).

The overall permeation of lidocaine from the mixtures was higher than that of prilocaine. For example, at the lidocaine ratio of 0.58 ± 0.002, the flux of lidocaine was $1230.41 \pm 84.36\ \mu g\,cm^{-2}\,h^{-1}$ and at a similar ratio (0.6 ± 0.002, the flux of prilocaine was significantly lower ($991.21 \pm 90.92\ \mu g\,cm^{-2}\,h^{-1}$). This is due at least in part to the fact that lidocaine is inherently more permeable than prilocaine through a hydrophobic silicone membrane due to its physicochemical properties. Permeability coefficients (K_p) calculated for lidocaine and prilocaine through human skin using the Potts and Guy equation (Potts and Guy, 1992) were found to be $1.01 \times 10^{-6}\ cm\,s^{-1}$ and $0.71 \times 10^{-6}\ cm\,s^{-1}$, respectively. Although these K_p values were predicted for skin, they allow us to interpret the silicone membrane data for the two local anaesthetics as a good skin-membrane correlation has previously been demonstrated. (Garrett and Chemburk, 1968; Woolfson et al., 1988, 1998).

At the eutectic ratio (1:1), the flux of lidocaine was not significantly different from that of prilocaine (Table 1, $p > 0.05$, t-test) and was therefore lower than expected considering its higher permeability coefficient. The equivalent flux of the two agents cannot be explained given the current knowledge. Attempts to determine interactions between the two agents have to date been unsuccessful and further investigation is required to model this process. The dip in lidocaine steady-state flux at the eutectic ratio when compared to the other ratios of pure drugs resulted in the total flux of the two agents being lowest at the eutectic ratio although the decrease in mpt was the greatest (Fig. 2).

Previously published data, which employed a similar set up but delivering the drugs with an aqueous vehicle, showed a different permeation behaviour compared to the pure eutectics (Fiala et al., 2008). Enhancement ratios (ER) for the eutectic mixtures when compared to two drug-containing aqueous solutions showed that prilocaine permeation was increased by 1.8–2.6-fold (a statistically significant difference in the enhancement degree existed between the different drug ratios, $p \leq 0.05$, ANOVA, Table 1) and lidocaine permeation was increased by 2.9–4.1 fold (a statistically significant difference in the enhancement degree existed between the different drug ratios, $p \leq 0.05$, ANOVA, Table 1). The permeation enhancement degree for both lidocaine and prilocaine was at its highest when either drug was in the majority. It is important to note that TEMPE spray which is a product in phase III development by Plethora Solutions Plc (London, UK; www.plethorasolutions.co.uk) as a topical treatment for PE (premature ejaculation) contains the local anaesthetics lidocaine and prilocaine at a 3:1 ratio suspended in HFA 134a (Henry, 1999; Henry and Morales, 2003; Henry et al., 2008). This lidocaine to prilocaine ratio has not been tested in the current study; however, the trend of increased total steady-state flux at lidocaine high ratios (≥ 0.6) may explain the use of such ratio in the development of TEMPE spray; additional studies are required for confirmation.

If the eutectic permeation enhancement was simply a consequence of the change in vehicle between the two systems, i.e. switching from an aqueous buffer to pure drug mix, the ER should have been relatively constant. It was interesting that the ER rose

dramatically at the ratios where it was suggested that equilibrium between the solid and liquid state had not been achieved. One hypothesis is that this lack of equilibrium could have induced a state of pseudosupersaturation of the drug molecules surrounding the solid particles in the mixture. The thermodynamic activity of these molecules increases as a function of diminishing distance to the particles and therefore they have a higher drive for permeation when presented at the membrane interface. A similar phenomenon was previously reported with ibuprofen and terpene combinations which were prepared using the melting technique; the best ER was achieved when solid phase existed in the eutectic mixtures (Stott et al., 1998). Although testing lidocaine and prilocaine when the mixtures had not reached equilibrium could have resulted in distorting the data in the current study, the mixtures were allowed to equilibrate for the same period of time (24 h) and hence this should not affect the comparative validity of the data.

4.2. Investigation of the mechanism of enhancement

The permeation enhancement demonstrated by the eutectic mixtures over the aqueous binary solutions could be attributed to many factors, including drug release, partition, diffusivity and diffusion path length. These variables were tested separately in order to examine if they influenced the delivery of lidocaine and prilocaine from eutectic mixtures.

4.2.1. Drug release

Regenerated cellulose membrane is a porous freely permeable membrane that allows the passage of molecules up to 14,000 Da. (Fiala et al., 2008). It has been used extensively to measure drug release rates from topical formulations (Guy and Hadgraft, 1990; Shah and Elkins, 1995). Previous work had shown that lidocaine and prilocaine were not retained by regenerated cellulose membrane (Fiala et al., 2008), therefore drug release from the formulation in the absence of partitioning was measured. There was a marked increase in the steady-state flux of both lidocaine and prilocaine from the eutectic mixture (1:1 ratio) when compared to the same ratio formulated as a binary saturated solution in phosphate buffer: $359.19 \pm 29.19\ \mu g\,cm^{-2}\,h^{-1}$ vs. $270.26\ \mu g\,cm^{-2}\,h^{-1}$ ($p < 0.05$) for lidocaine and $518.30 \pm 42.91\ \mu g\,cm^{-2}\,h^{-1}$ vs. $274.12\ \mu g\,cm^{-2}\,h^{-1}$ ($p < 0.05$) for prilocaine (Fiala et al., 2008). This could be merely due to a greater access of drug molecules to the membrane in the case of the eutectic mixture. In the binary phosphate buffer solution, the vehicle molecules have the potential to the access of the drug molecules to the formulation-membrane interface on a competition basis and this could result in drug release being less efficient. Such an effect suggests that vehicle-free delivery is the optimal system for the lidocaine–prilocaine eutectic mixtures.

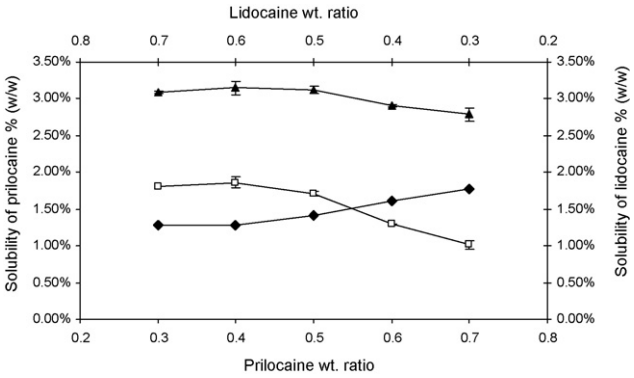

Fig. 3. Relationship between the maximum solubility of lidocaine and prilocaine in silicone membrane and the initial weight ratio in the eutectic mixture: prilocaine (♦), lidocaine (□) and total solubility (▲). Each point represents mean ± standard deviation ($n = 3$).

4.2.2. Silicone membrane solubility

The solubility of each drug in the silicone membrane was dependent on its ratio in the eutectic mixture (Fig. 3). The greater solubility when either drug dominates the mixture supports the competition theory described previously. However, because lidocaine is more lipophilic than prilocaine ($\log P$ −2.44 and 2.11, respectively, ChemIDplus database), it dominated membrane solubility when equal amounts of the two drugs were applied (Fig. 3). This superior membrane solubility did not translate to superior permeation of lidocaine compared to prilocaine as previously described (Fig. 2). These data further support the fact that the melting point theory previously suggested to explain eutectics permeation is perhaps too simplistic and that prilocaine and lidocaine might not be permeating as a complex structure through the silicone membrane.

The solubility of both lidocaine and prilocaine in silicone membrane was greater when applied as eutectic mixtures as opposed to binary saturated solutions (Fig. 4). This was suggested to be a result of the vehicle, i.e. water and phosphate molecules competing with lidocaine and prilocaine and thus reducing their solubility in the membrane when applied as saturated solutions. This is a very important finding as in a plethora of previous work in this area the solvent incorporated in topical preparations has been assumed to be inert. Treating the solvent as an inert component of the system is clearly not supported by the data arising from this study.

4.2.3. Effects on the membrane thickness

The change in membrane thickness after the application of either saturated solutions (binary and individual) or the eutectic mixture was not significant ($126.14 \pm 7.22\ \mu m$ at the start, and $126.17 \pm 7.68\ \mu m$ at the end of the permeation study, $p > 0.05$, t-test) indicating that none of the test systems caused significant

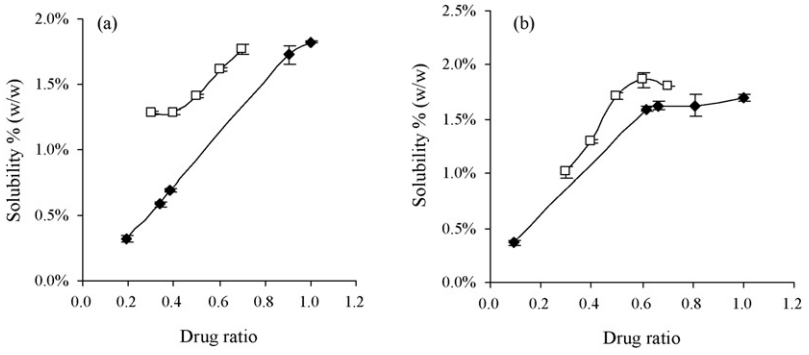

Fig. 4. Maximum solubility of prilocaine (a) and lidocaine (b) in silicone membrane when applied as a binary saturated solution in phosphate buffer (♦) or a binary eutectic mixture (□). Each point represents the mean ± standard deviation ($n = 3$).

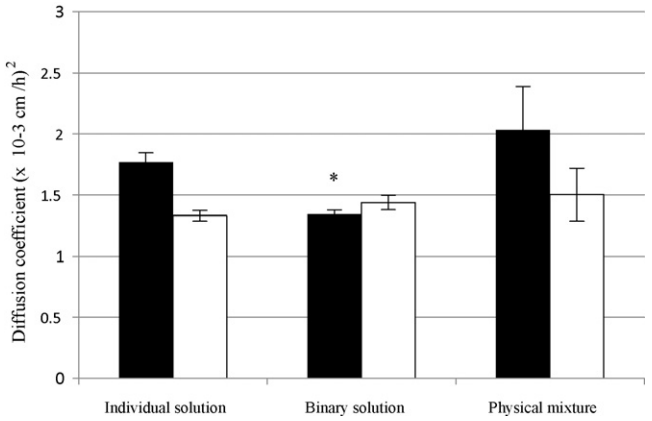

Fig. 5. Diffusion coefficient of prilocaine (■) and lidocaine (□) in silicone membrane using different formulations: prilocaine saturated solution, 8.60 mg ml^{-1} and lidocaine saturated solution, 3.64 mg ml^{-1}; binary saturated solution containing 3.36 mg ml^{-1} prilocaine and 2.47 mg ml^{-1} lidocaine; physical mixture containing 0.42% prilocaine and 0.58% lidocaine in the liquid phase. Each bar represents mean ± standard deviation ($n = 5$). *Significant difference from the individual solution.

swelling of the silicone membrane. A change in membrane thickness, hence path length could not be the reason for the greater steady-state flux observed with the eutectic mixtures.

4.2.4. Diffusivity

When the individually saturated phosphate buffer solutions were applied to the membrane, prilocaine diffused through the membrane faster than lidocaine (Fig. 5), which could be explained by its smaller molecular size (Mw −220 vs. 234, respectively). The diffusion coefficient of prilocaine was significantly reduced when applied as a binary saturated solution ($p < 0.05$, Fig. 5). Unfortunately, only one ratio was tested, therefore the effect of prilocaine to lidocaine ratio on the diffusivity of prilocaine could not be elucidated. Interestingly, when applied as a eutectic mixture, the diffusion coefficient of prilocaine ($2.03 \pm 0.34 \times 10^{-3}$ cm^2 h^{-1}) was significantly higher than that obtained using the aqueous binary solutions ($1.34 \pm 0.03 \times 10^{-3}$ cm^2 h^{-1}, $p < 0.05$, Fig. 5). This could be another reason for the enhanced flux of eutectic mixtures compared to aqueous binary solutions. Conversely, lidocaine diffusivity was not affected by the formulation or by the presence of prilocaine ($D_{lido} = 1.42 \pm 0.14 \times 10^{-3}$ cm^2 h^{-1}). This could be due to the fact that unlike prilocaine, lidocaine permeation was mostly affected by the partition process and not the diffusion process.

5. Conclusions

The permeation of lidocaine and prilocaine from eutectic mixtures was dependent on the drug composition of the mixture which indicated that competition between the transport of the two agents into the hydrophobic membrane was critical to the rate transmembrane transport. Lidocaine mass transport was most heavily influenced by the partition process and prilocaine by the diffusion process. However, at the (1:1) eutectic ratio, these competitive processes equilibrate to give equivalent fluxes for the two drugs.

When a vehicle such as phosphate buffer was used to administer the two drugs, the steady-state flux and solubility in silicone membrane were reduced for both agents. This was a significant finding as it shows that topical vehicles cannot be assumed to be inert in the case of eutectics and that the competitive transmembrane transport processes should be considered when such systems are used for drug delivery.

Pure eutectic mixtures of lidocaine and prilocaine do not appear to reach equilibrium in terms of their phase behaviour over 24 h. Maximal permeation was achieved with compositions where equilibrium was not reached between the solid and liquid phases. This discovery could imply that prilocaine and lidocaine were at a pseudo supersaturated state as a consequence of eutectic formation. However, this raises additional question surrounding formulation stability which could be a problem if the eutectic system is preformed prior to administration.

Acknowledgment

The authors would like to thank the Algerian government for the financial support of this work.

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