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An investigation into the influence of binary drug solutions upon diffusion and partition processes in model membranes

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Abstract

Few studies have assessed the impact of binary systems on the fundamental mathematical models that describe drug permeation. The aim of this work was to determine the influence of varying the proportions of prilocaine and lidocaine in a binary saturated solution on mass transfer across synthetic membranes. Infinite-dose permeation studies were performed using Franz diffusion cells with either regenerated cellulose or silicone membranes, and partition coefficients were determined by drug loss over 24 h. There was a linear relationship between the flux of prilocaine and lidocaine through regenerated cellulose membrane ($R^2 \geq 0.985$, n = 5) and their normalised ratio in solution. This linear model was also applicable for the permeation of prilocaine through silicone membrane ($R^2 = 0.991$, n = 5), as its partition coefficient was independent of the drug ratio (15.84 ± 1.41). However, the partition coefficient of lidocaine increased from 27.22 ± 1.68 to 47.03 ± 3.32 as the ratio of prilocaine increased and this resulted in a non-linear relationship between permeation and drug ratio. Irrespective of the membrane used, the permeation of one drug from a binary system was hindered by the presence of the second, which could be attributed to a reduction in available membrane diffusion volume.

Introduction

The stratum corneum, which is the uppermost layer of the epidermis, represents the main barrier to drug permeation into the skin (Michaels et al 1975). It has a ‘brick and mortar’ structure, with the corneocytes of hydrated keratin forming the ‘bricks’, which are embedded in a ‘mortar’ composed of multiple lipid bilayers of ceramides, fatty acids, cholesterol and cholesterol esters (Barry 2001). Although the structure of the stratum corneum is very heterogeneous, it can be considered as an inert barrier into which molecules partition and then penetrate passively according to Fick’s laws of diffusion (Barry 1999).

Assuming that diffusion in the skin is occurring under sink conditions, the amount of drug penetrating per time unit at steady state (dq/dt), often termed flux, is related to the surface area available for diffusion (A), the diffusion coefficient of the drug (D), its thermodynamic activity in the vehicle (α), its effective activity coefficient in the barrier phase (γ_{bar}) and thickness of the barrier (L). Thus, flux is proportional to the thermodynamic activity of the compound in the vehicle and not its concentration (Equation 1) (Higuchi 1960).

$$dq/dt = A(D/\gamma_{bar})(\alpha/L) \quad (1)$$

According to this relationship, commonly known as Higuchi’s equation, the flux of a compound from a saturated solution is constant, regardless of the saturated concentration in a given vehicle, because all saturated solutions have a thermodynamic activity of 1 (Twist & Zatz 1986). However, this mathematical model was designed to describe the mass transfer of a single agent and, even though the amount of topical products that include two therapeutic agents is increasing, the influence of two permeating species upon diffusion and partition from their formulations has yet to be systemically investigated.

Synthetic membranes have been found to be ideal for testing topical formulations without having to acquire human or animal skin (Sang-Chul & Soo-Young 1996; Pellett et al 1997b). These membranes have been used to study both transdermal (Fang et al 1999) and dermal (Sinico et al 2005) drug permeation, and examine such phenomena as ion-pairing (Trotta

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et al 2003), supersaturation (Hou & Siegel 2006), viscosity (Ruiz Martinez et al 2007), and particle size (Verma et al 2003), among others. Cellulose membranes, traditionally used for dialysis (Craig & Konigsberg 1961), have more recently been applied to measure drug diffusion (Loftsson et al 2002), to determine drug release rates from topical formulations (Guy & Hadgraft 1990) and to screen such formulations as ointments (Shah & Elkins 1995), creams (Shah et al 1992) and hydrogels (Wang et al 2001). A porous regenerated cellulose membrane (RCM) can be used to measure diffusion in the absence of partition. However, in order to study both processes that contribute to mass transport, a confluent membrane such as silicone can be used (Cappel & Kreuter 1991; Maitani et al 1995; Pellett et al 1997a; Müller & Kreuter 1999; Valenta et al 2000; Du Plessis et al 2001, 2002; Dias et al 2003). Although results of diffusion in RCM and mass transport across silicone cannot be directly compared, strategic use of both types of membranes can deconvolute diffusion and partition processes that influence specific drug compounds. Despite this, few previous studies have used model membranes to investigate dual drug penetration.

The enhancement potential of binary drug mixtures in topical delivery is well exemplified by the lidocaine–prilocaine system, which forms a eutectic in the solid state and dramatically increases the rate at which the two drugs permeate (Nyqvist-Mayer et al 1986). However, the mechanism by which this occurs has yet to be elucidated. This may be due to the lack of systemic investigation into the effects of using two drugs in a simple mass transport experiment. Thus, the aim of this work was to investigate the permeation behaviour from a series of lidocaine–prilocaine saturated aqueous solutions, and to determine the effects of applying two drugs on the processes of diffusion and partitioning into simple membranes. Using this study design, the applicability of Higuchi's equation to the permeation process of binary drug systems was assessed. Ultimately, it was anticipated that using this knowledge, a greater understanding of the enhancement potential of the eutectic physical mixture could be achieved.

Materials and Methods

Materials

Lidocaine was supplied by QueMaCo (Nottingham, UK) and prilocaine was purchased from Chemos GmbH (Regenstauf, Germany). Phosphate-buffered saline (PBS, pH 7.3, 0.172 M) tablets were supplied by Oxoid Ltd (Basingstoke, UK). Methanol HPLC grade was purchased from Fisher Scientific (Leicestershire, UK). Deionised water was obtained by purification using an Elgstat water purifier (Option 3A; Elga Ltd, Buckinghamshire, UK). Visking dialysis membrane (regenerated cellulose with a cut-off of 14 000 Da) was purchased from Medicell International Ltd (London, UK). Silicone membrane (Folioxane C6) with a thickness of 0.12 mm was obtained from Novatech Ltd (Cedex, France). Cellulose acetate syringe filters (GyroDisc CA-PC 30 mm, pore size 0.2 μm) were purchased from Orange Scientific (Braine-l'Alleud, Belgium).

Preparation of donor solutions

The individually saturated solutions were prepared by adding excess lidocaine or prilocaine to phosphate buffer (0.172 M, final pH 9.43 ± 0.13). The samples were stirred for 24 h at 25°C and filtered using 0.2-μm cellulose acetate syringe filters (recovery was > 99%). Saturated solutions containing both lidocaine and prilocaine were prepared by dissolving different amounts of a single compound, either lidocaine or prilocaine, in PBS (0.172 M, final pH 9.57 ± 0.09) and then adding the second compound to excess. The objective was to obtain saturated solutions with different concentrations of the individual drugs. The solutions were stirred for 24 h at 25°C (the saturated solubility was tested at 2, 8, 12, 24 and 48 h, and the solution reached equilibrium at 24 h; data not shown) and filtered using 0.2-μm cellulose acetate syringe filters. Concentrations of lidocaine and prilocaine in the donor solution were determined after saturation using high performance liquid chromatography (HPLC). As the pH range fell outside the buffering capacity of the phosphate, the pH of the saturated solutions was monitored throughout the Franz cell experiments and found to be over the range of 9.51 ± 0.13, which resulted in approximately 97% of prilocaine and lidocaine being in the unionised form (pKa for lidocaine 8.01 and prilocaine 7.89; ChemIDplus database, Bethesda, MD, USA). The variation in pH was relatively low (CV = 1.3%), and therefore it was assumed that the ionisation of the compounds remained unchanged across all the experiments. The eutectic mixture was prepared by the combination of 49.6% (w/w) lidocaine and 50.4% (w/w) prilocaine in the solid state (Brodin et al 1984). The mixture instantaneously melted and was stirred overnight using a magnetic stirrer until a clear liquid was obtained.

Franz diffusion cell studies

Unjacketed, individually calibrated, upright Franz diffusion cells, with surface areas of approximately 2.2 cm^2 and receiver compartment volumes of approximately 9.5 mL, were used for the permeation experiments. The regenerated cellulose membrane was prepared by soaking in hot water (60–70°C) for 1 h to remove the glycerine coating. The silicone membrane was used as obtained. The membrane was mounted between the donor and receiver chambers, which were sealed together using parafilm. Small magnetic bars were inserted into each of the receiver compartments to ensure adequate mixing and maintenance of sink conditions. The cells were inverted, the receiver compartments filled with PBS and each cell was checked for leaks. The assembled Franz cells were placed in a 25°C water bath (temperature selected to maintain the saturated solutions at equilibrium) and allowed to equilibrate for 30 min before use. Aliquots of 1.5 mL of the saturated solutions or eutectic mixture were added to the donor chambers to provide an infinite dose. At specified time intervals over a period of 5 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 performed. A minimum of four or five diffusion cells were used for each experiment and no cells were rejected throughout the study; cell rejection was

based on Box Plot outlier identification (Braun et al 2006). Cumulative amounts of drug (μg) penetrating the membrane per unit diffusional surface area of regenerated cellulose or silicone membrane (cm^2) were corrected for previous sample removal and plotted against time (h). The steady state flux (J_{ss}) was taken as the slope of the cumulative drug versus time plot in the linear region ($R^2 \geq 0.99$), which was over the range of 0.25–1.5 h (at least 5 time points).

Partitioning study

Solutions with different ratios of lidocaine and prilocaine were prepared by dissolving different amounts of lidocaine and prilocaine in PBS (pH 7.3, 0.172 M). Silicone membrane was cut into squares of 1.5 cm and placed into a vial to which 1 mL of the solution was added. The vials were agitated in a 25°C shaking water bath at a rate of 150 strokes min^{-1}. The amount of prilocaine and lidocaine in each solution was assayed by HPLC at t = 0 h and t = 24 h (time to reach equilibrium was determined by previous experiments; data not shown). The amount of the two drugs in the membrane was assumed to be equivalent to the difference in their concentration at t = 0 and t = 24. The partition coefficient of each drug was calculated using Equation 2:

$$P = Cm/Cv \quad (2)$$

where P is the partition coefficient, Cm is the concentration of drug in the membrane and Cv is the concentration of drug in the vehicle.

HPLC analysis

A liquid chromatography pump (Severn Analytical, Macclesfield, Cheshire, UK) with an AS 1000 autosampler (Spectra-Physics, Thermo Separation Products, Stone, Staffordshire, UK) connected to a UV 1000 absorbance detector (Spectra-Physics), was used for the quantitative determination of lidocaine and prilocaine. The HPLC system was connected to a computer with Chromeleon software (Dionex, Camberley, Surrey, UK), which was used to record and analyse the chromatograms. A Phenomenex Gemini C18 (5 μm, 250 × 4.6 mm) column (Phenomenex, Macclesfield, Cheshire, UK) was used with a 70:30 methanol/water mobile phase at pH 10.0 and a flow rate of 1 mL min^{-1}. The pH was selected so that > 99.9% of both drugs were in their unionised forms. Volumes of 10 μL were injected onto 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, Hengoed, Mid Glamorgan, UK). The method was previously shown to be fit for the 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).

Statistical analysis

Statistical evaluation was carried out using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). The effects of time (over the linear region of 0.25–1.5 h), membrane type and drug on the cumulative amount of the drug permeating to the receiver fluid were analysed using repeated measures analysis of variance. The effects of varying prilocaine and lidocaine ratios in the donor solutions on their permeation through both RCM and silicone membranes as well as their partition coefficients into silicone membrane were analysed statistically by one-way analysis of variance. Post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. A *t*-test was used to compare the steady state fluxes of prilocaine and lidocaine as well as to compare permeation through RCM and silicone membrane. A statistically significant difference was defined as $P \leq 0.05$. All values were expressed as mean ± s.d. The number of replicates was 4–5 in permeation studies and 3–5 in partition studies.

Results and Discussion

Diffusion through regenerated cellulose membrane

Both lidocaine and prilocaine displayed steady state mass transfer through the RCM over the first 1.5 h of the experiment ($R^2 > 0.99$, this was deemed as the steady state portion of the profile), therefore RCM did provide a rate-limiting barrier (Figure 1). Cellulose membranes have been proposed as porous inert membranes that can be used as a simple support scaffold for drug release studies (Barry & Brace 1977). If this was true, total equilibration of the donor and receiver solutions would be expected to occur rapidly in a similar manner to two solutions simply mixing. Yet, water is a powerful swelling agent for cellulose. It breaks the H-bonds within cellulose, expands the amorphous regions and increases the chain segmental motion (Tuwiner 1962; Reid et al 2008). Therefore, it was hypothesised that RCM created a hydrophilic unstirred layer in the system that acted as a barrier through which the two compounds diffused (Reid et al 2008). Prilocaine diffused through the RCM more rapidly compared with lidocaine ($P \leq 0.05$) (Table 1). This is probably due to differences in the physicochemical properties of the two compounds dictating their diffusivity through this porous hydrophilic barrier. Prilocaine is both smaller (MW 220) and more hydrophilic (log P 2.11, ChemIDplus database) than lidocaine (MW 234, log P 2.44, ChemIDplus database) and so it is not surprising that the former diffuses through RCM more rapidly.

In addition to the assumption that only one solute is moving through a barrier, Higuchi's equation (Equation 1) makes four further assumptions with regard to the environment in which mass transfer through a barrier is taking place: (i) the rate-controlling barrier is the membrane through which the agent has to pass; (ii) the application vehicle does not alter the barrier; (iii) the thermodynamic activity of the drug is homogenous throughout the vehicle; and (iv) mass transfer occurs under sink conditions. Ideally, if the effects of a second solute on the mass transfer process are going to be interrogated using Higuchi's equation, the test system used to investigate this process should satisfy these four assumptions.

RCM was specifically selected to allow the construction of a mass transfer experiment that obeys each of Higuchi's assumptions. Although porous membranes provide low resistance to the mass transfer of small molecular weight organic species such as lidocaine and prilocaine, previous work and the results of the present study have shown that such membranes do provide a rate-limiting barrier and thus the first of Higuchi's four assumptions was satisfied (Garrett & Chemburkar 1968; Reid et al 2008). RCM has excellent chemical resistance to a wide range of solvents (Reid et al 2008). In addition, neither lidocaine nor prilocaine partition into RCM (no loss of drug from applied saturated solutions was observed using a HPLC method with a limit of detection of 4.59 μg mL^{-1} and 13.34 μg mL^{-1}, for lidocaine and prilociane, respectively). As a result, the barrier should have remained constant across all the experiments, satisfying the second of Higuchi's four assumptions. The drug-saturated PBS solutions with different concentrations of prilocaine and lidocaine were allowed to reach equilibrium over 24 h and thus thermodynamic activity was maintained (equilibrium was confirmed by a solubility versus time study, which showed constant drug solubility after 12 h; data not shown). As a result, although exact control over the ratio of the compounds in solution could not be achieved due to the preferential precipitation of the agents, the thermodynamic activity was homogeneous in the donor chamber, which satisfies the third of Higuchi's assumptions. The drug concentration in the receiver chamber of the Franz cells did not exceed 10% of the maximum solubility of the compounds in the vehicle during the experiments, which means that sink conditions were maintained throughout the experiment, satisfying the last of Higuchi's assumptions. This test system was therefore deemed appropriate to determine the influence of the presence of two solutes on the mass transfer process.

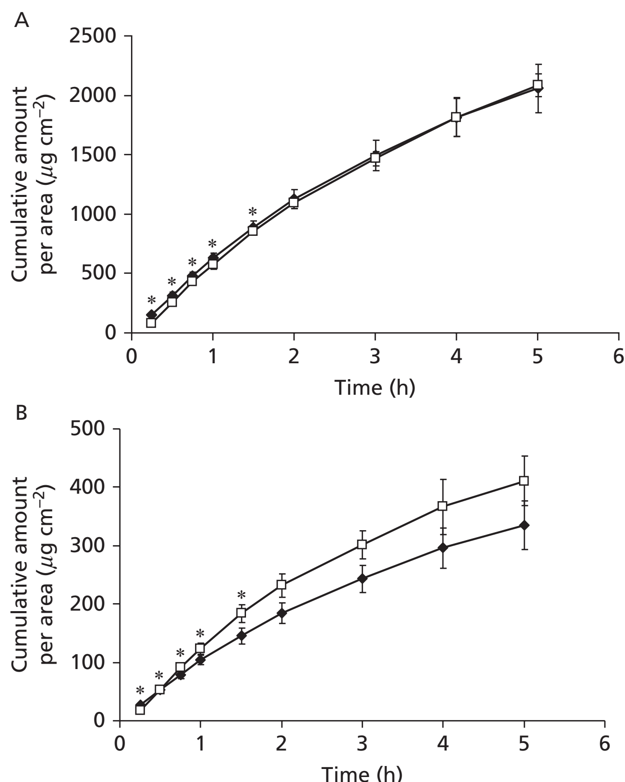

Figure 1 A. Permeation of prilocaine through regenerated cellulose membrane (♦) and silicone membrane (□) from binary saturated phosphate buffer solution containing 5.95 mg mL^{-1} prilocaine and 0.98 mg mL^{-1} lidocaine. Each point represents mean ± 1 s.d., n = 5. *$P \leq 0.05$, significant difference between time points (Tukey's HSD test). B. Permeation of lidocaine through regenerated cellulose membrane (♦) and silicone membrane (□) from binary saturated phosphate buffer solution containing 5.95 mg mL^{-1} prilocaine and 0.98 mg mL^{-1} lidocaine. Each point represents mean ± 1 s.d., n = 5. *$P \leq 0.05$, significant difference between time points (Tukey's HSD test).

If Highuchi's equation was applicable to model the mass transport of two solutes from a binary prilocaine–lidocaine solution through the RCM, then the flux from the saturated systems used in the current study should be constant. However, statistical analysis showed a significant difference ($P \leq 0.05$, analysis of variance) between the steady state fluxes of prilocaine and lidocaine from binary solutions across the different concentrations tested (Table 1). As the ratio of prilocaine or lidocaine increased in the donor solution, the steady state flux of that agent consequently increased (Table 1).

The mass transfer experiment using RCM was designed not only to satisfy Higuchi's four assumptions, but also to

Table 1 Permeation of lidocaine and prilocaine through regenerated cellulose membrane

	Prilocaine		Lidocaine	
	Concentration in the donor fluid (mg mL^{-1})	Steady state flux (μg cm^{-2} h^{-1})	Concentration in the donor fluid (mg mL^{-1})	Steady state flux (μg cm^{-2} h^{-1})
Lidocaine saturated solution (n = 5)	NA	NA	4.09 ± 0.15	404.8 ± 21.2
Solution 1 (n = 5)	0.94 ± 0.09	95.2 ± 5.5*	3.60 ± 0.34	366.1 ± 22.3
Solution 2 (n = 5)	1.96 ± 0.03	184.0 ± 23.0	3.87 ± 0.03	352.9 ± 41.7
Solution 3 (n = 5)	2.85 ± 0.13	255.8 ± 45.2	3.18 ± 0.08	276.7 ± 42.5*
Solution 4 (n = 5)	5.95 ± 0.21	589.7 ± 39.5*	0.98 ± 0.09	94.4 ± 9.1*
Prilocaine saturated solution (n = 5)	7.18 ± 0.73	708.7 ± 71.0*	NA	NA
Eutectic mixture (n = 5)	502.24	518.3 ± 42.9	494.26	359.2 ± 29.2

NA, not applicable. Each number represents the mean ± 1 s.d., n = 5. *$P \leq 0.05$, significant difference between the groups (saturated solutions only) (Tukey's HSD test).

specifically assess the impact of applying two solutes in a single vehicle on the membrane diffusion of the individual solutes. This was achieved by standardising each of the other parameters that could influence mass transfer according to Higuchi's equation. These parameters were: (i) area, which was corrected for in the measurement of flux; (ii) activity coefficient, which was negligible as the drugs were not retained by the membrane (indicated by partitioning studies); (iii) thickness of the RCM, which remained constant (the same donor and receiver vehicles were used throughout); and (iv) thermodynamic activity, which was always 1 (saturated solutions were always applied). As a result, it was hypothesised that a change in membrane diffusion was responsible for the unequivalent fluxes observed in the RCM experiments, however as no permeation lag time could be measured, this could not be accessed directly. The alteration in mass transfer rate could theoretically be due to the compounds interacting in the donor solvent and thus diffusing as a single species. However, previous work has shown that this is not the case. The self-diffusion coefficient, that is the diffusion coefficient of lidocaine and prilocaine in the vehicle both individually and in combination ($\sim 7.5 \times 10^{-6}$ cm^2 s^{-1}), was previously found to be equivalent in an aqueous environment (Nyqvist-Mayer et al 1986). This suggests the absence of interactions between the two compounds and/or the solvent and therefore this is not the reason why the inclusion of a second compound reduces diffusion rate of the first through RCM.

An alternative hypothesis for the change in the steady state flux of the two compounds could be a reduction in the capacity of the membrane to allow unhindered diffusion of one compound in the presence of the second. This reduction in membrane diffusion area or volume could simply be caused by the presence of a second agent occupying 'space' at the interface or in the membrane. Further spectroscopic studies to determine the relative proportions of the two compounds in the membrane could be used to interrogate this explanation further, however using current analytical technology this may be practically challenging.

Regardless of the exact mechanism by which the rate of mass transfer through the RCM is changing, it appears that in a binary system, the rate of diffusion is dependent on the proportion of the two compounds present in the system. Hence, Higuchi's equation cannot be used to model this mass transfer process in its current form, but it can be adapted to account for this effect. However, a complicating factor in defining the ratio of the two agents in the vehicle is that the solubility of one agent is affected by the second (Table 1). For example, although lidocaine has a solubility of 4.09 ± 0.15 mg mL^{-1} (0.017 M) in the aqueous donor fluid used in this study, the addition of 0.94 ± 0.09 mg mL^{-1} (0.004 M) prilocaine reduced this solubility to 3.60 ± 0.34 mg mL^{-1} (0.015 M). This corresponds to a total solid content of approximately 4.56 mg mL^{-1} (0.019 M), which is higher than the initial pure lidocaine content of the solution. As a consequence, when saturating a solution with the two compounds, the total amount is dependent on the proportion of the two compounds in solution. Hence, using a simple mass ratio of the two agents in an attempt to determine the relationship between diffusion coefficient and flux is inappropriate. Therefore, a normalised ratio that takes into account the amount of each agent present in the system as a function of solubility was used to investigate the influence of the RCM diffusion coefficient on flux (Equations 3 and 4):

$$N_{prilo} = C_{prilo}/(C_{prilo} + (C_{lido} \times S_{prilo}/S_{lido})) \quad (3)$$

$$N_{lido} = C_{lido}/(C_{lido} + (C_{prilo} \times S_{lido}/S_{prilo})) \quad (4)$$

where S_{prilo} and S_{lido} are the solubility of prilocaine and lidocaine in individually saturated solutions, respectively; C_{prilo} and C_{lido} are the concentrations of prilocaine and lidocaine in the binary solution, respectively; and N is the normalised ratio.

The steady state flux from PBS solutions was plotted against the normalised ratios and a linear relationship was observed (R^2 = 0.9963 for prilocaine and 0.9849 for lidocaine, n = 5, Figure 2). The fact that the reduction of the permeation rate of one compound was proportional to the amount of the second compound supports the hypothesis that the most likely explanation for the reduction of mass transport through RCM using a binary system is due to an alteration in available membrane diffusion volume as a consequence of two diffusing species. If the normalised ratio is added to Equation 1, the diffusion coefficient of the two compounds in the RCM is constant and re-arrangement of the equation allows the calculation of the true membrane diffusion coefficient of each compound (assuming activity coefficient in the barrier is negligible, Equation 5). For lidocaine this was 5.08 ± 0.22 cm^{-1} and for prilocaine this was 9.08 ± 0.50 cm^{-1}.

$$dq/dt = A(ND/\gamma_{bar})(\alpha/L) \quad (5)$$

Interestingly, the steady state flux of lidocaine from the eutectic mixture through RCM (359.2 ± 29.2 μg cm^{-2} h^{-1}) was significantly greater than that predicted from the equivalent ratio mixture formulated in PBS (270.3 μg cm^{-2} h^{-1})

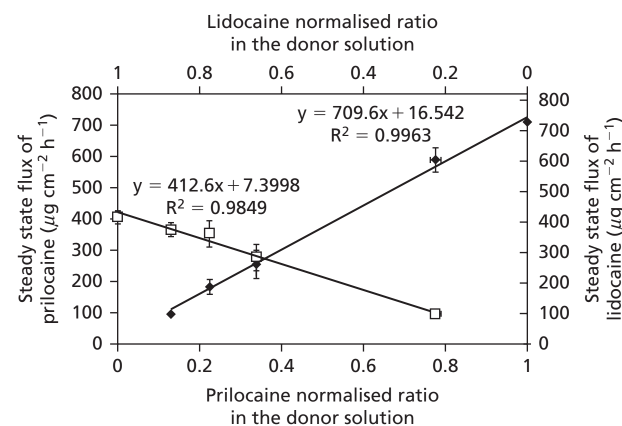

Figure 2 Relationship between the steady state flux of lidocaine and prilocaine through regenerated cellulose membrane and their solubility normalised ratio in phosphate buffer solution: prilocaine (♦), lidocaine (□). Each point represents mean ± 1 s.d., n = 5. A linear trend was observed between the steady state flux of prilocaine and lidocaine and their solubility normalised ratios in the donor solution and was plotted (represented by a black line).

Table 2 Permeation of lidocaine and prilocaine through silicone membrane

	Prilocaine		Lidocaine	
	Concentration in the donor fluid (mg mL^{-1})	Steady state flux ($\mu g\ cm^{-2}\ h^{-1}$)	Concentration in the donor fluid (mg mL^{-1})	Steady state flux ($\mu g\ cm^{-2}\ h^{-1}$)
Lidocaine saturated solution (n = 5)	NA	NA	4.09 ± 0.15	523.1 ± 23.9
Solution 1 (n = 5)	0.94 ± 0.09	91.7 ± 9.8*	3.60 ± 0.34	491.4 ± 38.8
Solution 2 (n = 5)	1.96 ± 0.03	203.6 ± 14.7	3.87 ± 0.03	523.3 ± 36.1
Solution 3 (n = 5)	2.87 ± 0.12	289.3 ± 14.6	3.19 ± 0.07	420.9 ± 24.0*
Solution 4 (n = 5)	5.95 ± 0.21	617.1 ± 28.3*	0.98 ± 0.09	133.2 ± 12.6*
Prilocaine saturated solution (n = 5)	7.18 ± 0.73	725.5 ± 98.7*	NA	NA
Eutectic mixture (n = 4)	502.236	712.1 ± 39.4	494.26	762.7 ± 44.6

NA, not applicable. Each number represents the mean ± 1 s.d., n = 4–5. *$P \leq 0.05$, significant difference between the groups (saturated solutions only) (Tukey's HSD test).

($P < 0.05$). The enhancement ratio was 1.33. Similarly, the flux of prilocaine from the eutectic mixture (518.3 ± 42.9 $\mu g\ cm^{-2}\ h^{-1}$) was significantly higher than that predicted from a PBS solution (274.1 $\mu g\ cm^{-2}\ h^{-1}$) ($P < 0.05$). The enhancement ratio was 1.89. While it is accepted that the viscosity of the solutions and the eutectic may be different, the eutectic was still a free-flowing liquid and thus any influence that viscosity had on drug permeation was perceived to be minimal. It has been previously hypothesised that the decrease in melting point of the eutectic mixture was responsible for the increase in the drug solubility in skin lipids, which in turn provides a higher concentration gradient for permeation (Stott et al 1998). However, it is important to note that RCM is not a lipid membrane and therefore the increase in steady state flux cannot be due to an increase in the drug lipid solubility but is due to an enhanced release from the formulation caused by the absence of the aqueous vehicle.

Permeation through silicone membrane

In contrast to cellulose, the silicone membrane represents a non-porous hydrophobic barrier. It is important to note that as with the stratum corneum, silicone membranes, although hydrophobic in nature, still allow the passage of water (Valenta et al 2000). The purpose of using silicone in this study was to illustrate the permeation of lidocaine and prilocaine through a hydrophobic barrier where the permeation is governed by the processes of both partitioning and diffusion. This model is more realistic compared with the RCM when modelling the processes that occur during mass transfer across the stratum corneum. As a consequence, unlike when using the RCM, all of Higuchi's assumptions could not be satisfied in the experimental design. It was not certain that the properties of the membrane would not change as a result of the different ratios of the two drugs partitioning into the membrane and this became a second variable in addition to the available membrane diffusional volume that was potentially changing across the series of experiments.

The steady state flux of lidocaine through silicone membrane was 523.1 ± 23.9 $\mu g\ cm^{-2}\ h^{-1}$, which was significantly greater ($P \leq 0.05$, *t*-test) compared with the equivalent experiment using cellulose membrane (404.8 ± 21.2 $\mu g\ cm^{-2}\ h^{-1}$) (Figure 1). Although prilocaine permeated through the silicone membrane more rapidly compared with lidocaine, unlike lidocaine, the steady state flux of prilocaine did not increase significantly using the silicone membrane compared with the RCM ($P > 0.05$, *t*-test, Table 2). The increase in flux observed by lidocaine in the silicone membrane compared with RCM was coupled with the significantly higher partition coefficient of lidocaine (27.22 ± 1.68) when compared with prilocaine (15.51 ± 0.25). Again the behaviour of the two compounds in the model membranes can be explained in terms of their physicochemical properties. It was assumed that the higher log P of lidocaine drives the enhanced partitioning of the compound into the hydrophobic silicone membrane, but the smaller size of prilocaine maximises the diffusion of the compound through the membrane. These results indicate that molecular size is crucial to the rate at which lidocaine and prilocaine permeate through lipophilic barriers. Such findings are in agreement with previous workers who have demonstrated, using regression analysis on a set of experimental data, that molecular size is the dominant determinant of solute maximum flux across the skin (Magnusson et al 2004), whereas log P is mainly important for the determination of the permeability coefficient (k_p) (Potts & Guy 1992; Magnusson et al 2004).

In a similar manner to RCM, statistical analysis showed a significant difference ($P \leq 0.05$, analysis of variance) between the steady state fluxes of prilocaine and lidocaine from binary solutions across the different concentrations used (Table 1). The relationship between prilocaine steady state flux through silicone membrane and its normalised ratio in the solution was linear ($R^2 = 0.9907$, n = 5, Figure 3). This implies that the effects of adding lidocaine on the mass transfer of prilocaine through silicone are a consequence of a reduction in available membrane diffusion volume. The permeability coefficient was calculated for the individually saturated solution as well as the binary solutions 1–4 (Table 2) and was found to be constant (k_p(prilo) = 101.44 ± 10.01 cm h^{-1}, no significant difference, $P > 0.05$, analysis of variance). The lack of change in k_p in a binary solution should be interpreted with care, as the presence of a second agent means that the permeability of prilocaine through silicone membrane cannot simply be related to the applied concentration. In this study,

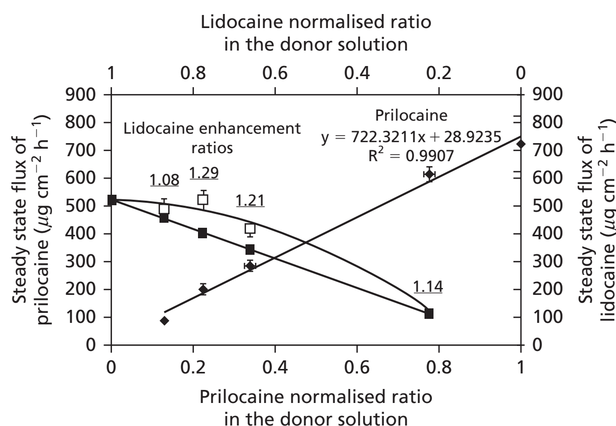

Figure 3 Relationship between the steady state flux of lidocaine and prilocaine through silicone membrane and their solubility normalised ratio in phosphate buffer solution: prilocaine (♦), lidocaine (□). Theoretical lidocaine flux (■) was calculated assuming that the diffusion volume was changing as a function of the normalised ratio. Each point represents mean ± 1 s.d., n = 5. Enhancement ratios of lidocaine were calculated as the ratio of the actual to the theoretical steady state flux and are indicated by the numbers on the graphs.

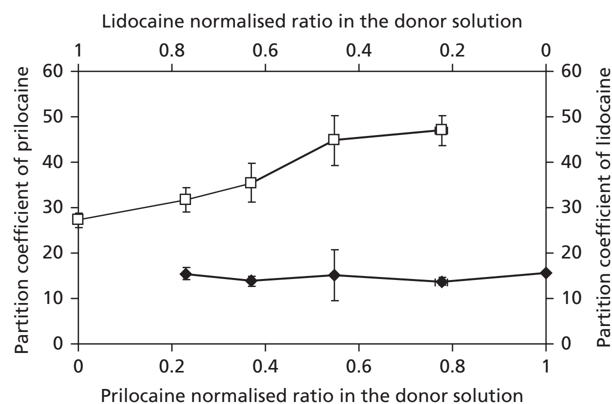

Figure 4 Relationship between silicone partition coefficient and the solubility normalised ratio of drug in solution for prilocaine (♦) and lidocaine (□). Each point represents mean ± 1 s.d., n = 3–5.

altering the concentration of one agent influenced its relative ratio with the second and therefore the access of the two compounds to the membrane, supporting the membrane volume hypothesis. The partition coefficient of prilocaine was also found to be independent of the drug ratio (no statistically significant change, $P > 0.05$, analysis of variance) (Figure 4) and this confirms that the change in permeation of this solute across the membrane in the presence of lidocaine was due to a change in the available membrane diffusion volume and not altered by the partitioning process.

A linear model could not be applied to the changes in permeation of lidocaine as a result of different prilocaine to lidocaine ratios (Figure 3). Using Equation 5, that is assuming the flux of lidocaine through silicone was influenced only by a reduction in available membrane diffusion volume, the theoretical flux of lidocaine through silicone membrane and enhancement ratios of the actual flux versus the theoretical flux were calculated and added to Figure 3. The greatest lidocaine permeation enhancement was observed at high normalised lidocaine ratios (i.e. > 0.6). As the enhancement ratio provided a measure of permeation above that expected from the effects of reduced diffusion volume due to the presence of two agents, the flux enhancement observed for lidocaine could be due to the partitioning behaviour, which was changed in the presence of prilocaine.

Pure lidocaine had a partition coefficient of 27.22 ± 1.68 and this increased to 47.03 ± 3.32 when the normalised ratio of lidocaine was reduced to approximately 0.2 (the differences in partition coefficients were found to be significant, $P \le 0.05$, analysis of variance). Using Fedors method (Fedors 1974), the solubility parameter of the silicone membrane was calculated as 7.3 (cal $cm^{-3})^{1/2}$, for lidocaine 10.68 (cal $cm^{-3})^{1/2}$ and for prilocaine 11.05 (cal $cm^{-3})^{1/2}$. Thus, it is not surprising that the partition of prilocaine into the membrane enhances the partition of lidocaine as it makes the membrane more hydrophilic, that is it increases the solubility parameter to make it more similar to lidocaine. This could also be true for prilocaine, as lidocaine renders the membrane more hydrophilic (although not to such a great extent as prilocaine) by increasing its solubility parameter to be more like prilocaine. However, this effect was not apparent for prilocaine because, unlike lidocaine, its permeation is mostly influenced by diffusion rather than partition.

Although the γ_{bar} for lidocaine was dependent on the ratio of the two compounds applied to the silicone, this does not explain why lidocaine permeated through the silicone membrane more rapidly than predicted by the corrected Highuchi equation because permeation enhancement did not correlate with partitioning enhancement (permeation enhancement ratios were lower when partitioning was highest). Hence, this phenomenon must be related to diffusion effects. Further work is required to test this hypothesis. Unfortunately, the permeation lag time in these experiments was less than about 5 min and was irreproducible (% CV > 100%). Therefore, it was not possible to calculate the diffusion coefficient for the compounds in these experiments. However, permeability coefficients of lidocaine were calculated and, interestingly, were found not to be significantly different with varying concentrations (k_p(lido) = 134.15 ± 12.02 cm h^{-1}, $P > 0.05$, analysis of variance). This is identical to the trend reported for prilocaine and again concurs well with the membrane diffusion volume hypothesis.

The trend of increased lidocaine permeation at high lidocaine ratios may be slightly distorted by the lack of test solutions with a normalised lidocaine ratio of between approximately 0.65 and 0.2. However, this was a consequence of the recrystallisation behaviour of the two agents in phosphate buffer solution. As such, using traditional saturation methods high prilocaine mixtures could not be achieved. To confirm if the deviation from the linear model was a result of partitioning, further work is required to develop a method to generate different binary solutions with higher prilocaine content. However assuming that this trend is confirmed, the enhanced lidocaine permeation must be as a

result of a higher diffusion coefficient through the membrane than predicted.

The permeation rate of both lidocaine and prilocaine from the pure eutectic mixture dramatically increased (762.7 ± 44.6 μg cm^{-2} h^{-1} and 712.1 ± 39.4 μg cm^{-2} h^{-1}, respectively) when compared with that expected from a phosphate buffer solution (430.4 μg cm^{-2} h^{-1} and 291.1 μg cm^{-2} h^{-1}, respectively) ($P \leq 0.05$) in a similar manner to the RCM. This is equivalent to an enhancement ratio of 1.77 and 2.45, respectively, which was significantly higher in silicone compared with the RCM ($P \leq 0.05$). This shows that the eutectic influences not only the release of the drug from the formulation (shown by the RCM studies), but also the partitioning due to the additional enhancement observed in the silicone membrane. Hence, the delivery of lidocaine and prilocaine using a eutectic system enhanced the permeation of the two drugs even when taking into account the effects of the dual drug application which was previously ignored.

Although the use of two different types of membranes (hydrophilic and hydrophobic) provided a useful means of studying different permeation behaviours, including drug release and partitioning from dual drug systems, these artificial membranes are very simplified models of the biological barriers. Therefore, the experimental methodology used in this work will be used to design in-vitro human skin studies in order to provide a more relevant and practical insight into mass transport from dual drug systems.

Conclusion

Higuchi's equation could be applied to lidocaine and prilocaine in a binary system using RCM if modified to account for the normalised ratios of the drugs in the saturated solution. This was necessary due to the influence of the two drugs on the available membrane diffusion volume. The use of silicone membrane made mathematical modelling much more complex due to the influence of drug partitioning. Although the partitioning of lidocaine was influenced by the quantity of prilocaine in the membrane, this did not explain the non-linear response between the normalised ratio of the two agents and their flux. The presence of prilocaine in the silicone membrane appeared to change its properties and this influenced the diffusion of lidocaine through it.

The permeation of the two species from a pure eutectic mixture of prilocaine and lidocaine resulted in a significantly enhanced rate of mass transfer using both RCM and silicone. Comparing this data with simple binary solutions allowed the mechanism of eutectic enhancement to be deconvoluted. The improved release in the RCM compared with the saturated solutions showed that the formation of a eutectic mixture enhanced drug release. Furthermore, the additional enhancement of mass transfer in the silicone membrane compared with the RCM for the eutectic showed that the enhanced partitioning of one or both of the agents was also a contributory factor to the efficiency of the eutectic system. The use of artificial membranes provided an insight into the fundamentals of dual drug permeation, which will be further reinforced by additional in-vitro studies using excised human skin.

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