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The influence of volatile solvents on transport across model membranes and human skin

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ABSTRACT

Simple topical formulations which include volatile components, such as gels or sprays, are appealing from a cosmetic perspective. However, complex formulation effects may result from the use of volatile excipients in topical formulations, particularly when applied at clinically relevant doses (typically less than a few mg cm^{-2}). The present investigation aims to study the role of the volatile solvent ethanol (EtOH), in combination with Transcutol P® (TC), dimethyl isosorbide (DMI) and isopropyl myristate (IPM), on the efficacy of dermal delivery of a model compound (i.e. methyl paraben). The methodology consisted of in vitro diffusion experiments conducted using silicone membranes and human epidermis. Finite dose studies were performed with two types of formulations: saturated solutions of methyl paraben in each vehicle alone and incorporating the volatile solvent in a 50:50 (v/v) proportion. The kinetics of EtOH evaporation from the formulations were also investigated by monitoring the weight loss of the formulation over time. The results showed that the presence of EtOH had little effect on the skin flux of methyl paraben compared with the corresponding saturated solutions. Formulations incorporating the volatile solvent were clearly more efficient, in line with the data obtained with silicone membranes. Furthermore, the permeation of methyl paraben from the saturated EtOH solution in both silicone and skin showed an initial period of relatively fast permeation, after which there was a marked decrease in the permeation rate. This reflected significant ethanol depletion from the formulation (chiefly by evaporation), causing most of the dose of methyl paraben applied to crystallise as a deposited film at the skin surface (observed experimentally and confirmed by mass balance studies), thus decreasing its availability to permeate. Studies of the kinetics of ethanol evaporation from the formulations confirm these findings, demonstrating a very short residence time of the volatile solvent at the surface of the membrane (approx. 6 min). In conclusion, the findings suggest that rapid evaporation of EtOH takes place from the formulations applied at the surface of the skin, leaving a saturated residue of the drug in the vehicle. The presence of EtOH clearly influenced the efficiency of the formulation, underlining the application of volatile components to optimise dermal delivery.

1. Introduction

Overcoming the excellent barrier properties of the human skin represents the major challenge and limitation to transdermal drug delivery. An informed choice of excipients is therefore the key to construct elegant yet efficient transdermal formulations.

Simple topical formulations which include volatile components, such as gels or sprays, are appealing from a cosmetic perspective. In the literature, solvent evaporation has been described as a technique to concentrate the solute and/or produce supersaturated states in the formulation, thus increasing permeation. Coldman et al. (1969) were the first to realise the potential of combining volatile and non-volatile vehicles for dermal delivery, in their investigation of the skin permeation of fluocinolone acetonide and its acetate ester from different isopropanol: propylene glycol and isopropanol: IPM co-solvent mixtures. The authors found significant penetration enhancement from formulations with increasing amount of volatile solvent, owing to increased thermodynamic activity of the solute in the residual phase (non-volatile vehicle). However, this effect was limited by the precipitation of the steroids from the supersaturated solutions, and was not observed when the evaporation of isopropanol was prevented (i.e. occluded conditions).

The use of penetration enhancers is a well-known strategy to improve delivery across human skin. Few studies, however, have looked at the effect of the enhancer when applied at clinically relevant doses (typically less than a few mg cm^{-2}). In this case, complex formulation effects may also result from the use of volatile excipients in topical formulations. Ultimately, drug transport will be a function not only of the type of excipient, but also of the ultimate

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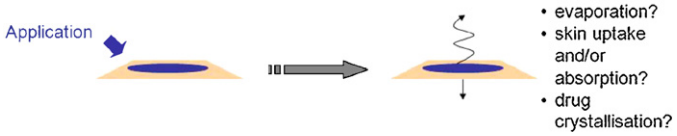

Fig. 1. Examples of possible dynamic processes occurring in formulations following topical application.

fate of the excipients after application (Fig. 1). Marked depletion of the vehicle from the formulation and skin, particularly when applied at finite doses, may cause the drug to precipitate; thus impacting on both the rate and extent of dermal absorption (Akhter and Barry, 1985; Chiang et al., 1989; Trottet et al., 2004; Santos et al., 2010, 2011).

The aim of the present investigation is to study the role of the volatile solvent ethanol (EtOH), in combination with Transcutol P® (TC), dimethyl isosorbide (DMI) and isopropyl myristate (IPM), on the efficacy of dermal delivery of a model compound (i.e. methyl paraben) when applied at clinically relevant doses. Complementary studies were also conducted in silicone membranes, including ethanol evaporation studies, to help elucidate the basic mechanisms of transport from the volatile formulations tested. Model membranes such as silicone offer a simple and reproducible alternative to study the basic mechanisms governing membrane transport in more complex biological tissue (Watkinson et al., 2009).

The ultimate objective of this work is to assess the importance of the residence time of excipients in the residual formulation and/or inside the skin on drug transport.

2. Materials and methods

2.1. Materials

Methyl paraben (methyl-4-hydroxybenzoate, puriss. ≥99%, Fluka) was supplied by Sigma–Aldrich UK. Ethanol (99.7–100% (v/v) AnalaR® grade, BDH) was supplied by VWR UK and IPM (isopropyl myristate 98%, Aldrich) was supplied by Sigma–Aldrich, UK. Dimethyl isosorbide (Arlasolve® DMI) and Transcutol (Transcutol P®) were supplied by Croda (Goole, UK) and Gattefossé (France), respectively. PBS was prepared in situ by dissolving 10 phosphate buffered saline (Dulbecco A) tablets (pH 7.3 ± 0.2 at 25 °C, Oxoid) supplied by Fisher Scientific UK in 1 L of deionised water (Elga Option 3 water purifier, ELGA LabWater, UK). Phosphate buffered saline (PBS) containing 0.002% sodium azide was prepared in situ by dissolving 10 PBS (Dulbecco A) tablets (pH 7.3 ± 0.2 at 25 °C, Oxoid) supplied by Fisher Scientific UK in 1 L of deionised water, and adding 1 mL of a 2% (w/v) sodium azide stock solution (sodium azide purum p.a. ≥99.0% (T), Fluka). Both receptor phases were degassed by high speed stirring under vacuum for ~20 min in a Nuova II stirrer (Thermolyne, US) connected to a vacuum pump. Silicone membranes were obtained from Samco (Nuneaton, UK). All solvents used in the HPLC analysis were HPLC grade and supplied by Fisher Scientific, UK.

2.2. Preparation of the formulations and solubility studies

Saturated solutions of methyl paraben in each vehicle were produced by adding an excess amount of the solute to each solvent in a glass vial with a Teflon coated magnetic flea. The containers were capped and sealed with Parafilm®, and placed inside a temperature controlled water bath. The system was allowed to equilibrate by stirring for at least 24 h at 32 (±0.5) °C to produce a saturated solution with visible excess chemical. The time to achieve saturation was investigated after 48 h equilibration using deionised water as a solvent. The results showed no significant difference ($p > 0.05$), thus 24 h was found to be sufficient time to achieve saturation. The saturated suspensions were then sampled and filtered using a syringe and filtration unit (13 mm PTFE filter media device, 0.2 μm pore size, Whatman®, UK) previously conditioned at the same temperature to avoid further precipitation/solubilisation of the drug, to produce the saturated solutions of methyl paraben in each solvent. Samples of the saturated solutions were suitably diluted using ethanol and quantified by high performance liquid chromatography (HPLC) with UV detection (Section 2.8) to determine the solubility of methyl paraben in each vehicle. No precipitation of methyl paraben was observed upon dilution of the solubility samples. Formulations incorporating the volatile solvent were also prepared using saturated solutions of methyl paraben and ethanol in a 50:50% (v/v) proportion. Table 1 lists the formulations used in this study and the respective doses applied for each formulation.

2.3. Permeation studies using silicone membranes

The permeation experiments were conducted at 32 (±1) °C using Franz-type diffusion cells (~0.80 cm^2 diffusion area, accurately measured for each Franz cell) placed in a temperature controlled water bath. Degassed PBS was used as receptor phase. The silicone membranes were cut to appropriate size and presoaked overnight in PBS at the experimental temperature before starting the experiment. The pre-treated membranes were carefully blotted with absorbent paper tissue to remove excess solvent before assembly in the Franz cells. High vacuum grease (Dow Corning®, US) and a metallic clamp were used to create a leak proof seal between donor and receptor compartments. The Franz cells were left inside the temperature controlled water bath for about 30 min before starting the experiment for temperature regulation. Finite dose experiments were conducted using small volumes (12.5 μL cm^{-2}) of the test formulations (Table 1), evenly spread on the membrane surface using a micropipette. Non-occluded conditions were used. A permeation experiment using neat methyl paraben powder as the donor in a finite dose equivalent to that after application of 10 μL of a saturated solution in EtOH (~30.5 μmol cm^{-2}) was also conducted to assess the permeation of the compound in the absence of liquid solvent. The pre-weighed powder was carefully spread over the surface of the membrane as evenly as possible using a metal spatula and the donor compartment was occluded to avoid the accumulation of moisture from the atmosphere. The receptor phase was continuously stirred with a small Teflon coated magnetic flea. Sampling occurred at designated time points with volume replacement using fresh receptor solution. Sink conditions were maintained throughout the experiment. At the end of all experiments, the membranes were carefully blotted and the thickness was measured using an electronic outside micrometer (RS Components Corby, UK; 0–25 mm, min 0.001 mm). The concentration of methyl paraben in all samples and formulations was quantified using HPLC with UV detection (Section 2.8).

2.4. Studies of ethanol evaporation from formulations

The kinetics of ethanol evaporation from formulations incorporating volatile solvent were studied by monitoring the weight loss of the formulations over time. A finite volume of the formulations (12.5 μL cm^{-2}) was evenly applied to the surface of previously weighed silicone membrane discs using a micropipette, which were placed in a balance (Sartorius balance, ±0.0001 g accuracy). The experiment started after the initial weight of the formulation was recorded, once the balance readings stabilised ($t = 0$), and the weight loss was recorded at regular intervals over a period of 10 min. The time required for balance reading stabilisation was approximately 10 s. Controls on glass cover slips were also

Table 1
Formulations tested in the finite dose studies and respective average dose of methyl paraben applied in model membranes and human skin (MP, methyl paraben; IPM, isopropyl myristate; DMI, dimethyl isosorbide; TC, Transcutol P®; EtOH, ethanol; and sat., saturated solution).

Formulation	Composition	Dose applied ($\mu mol\,cm^{-2}$)
MP in IPM sat.	Saturated solution of MP in IPM	2.5
MP in DMI sat.	Saturated solution of MP in DMI	22.2
MP in TC sat.	Saturated solution of MP in TC	30.4
MP in EtOH sat.	Saturated solution of MP in EtOH	25.6
IPM sat. + EtOH (50:50)	MP in IPM sat. (500 μL) + EtOH (500 μL)	1.4
DMI sat. + EtOH (50:50)	MP in DMI sat. (500 μL) + EtOH (500 μL)	11.6
TC sat. + EtOH (50:50)	MP in TC sat. (500 μL) + EtOH (500 μL)	14.4

measured for 100% ethanol and for the IPM based formulation (solvent with highest membrane uptake). The measurements were conducted at room temperature (~26 °C). Balance readings were considered stable when differing in less than ±0.0002 g (i.e. ~3% of the initial weight) over a period of 4 min. The results were presented in weight percentage (%), normalised for the initial weight of the formulations in grams.

2.5. Preparation of human epidermal skin membranes

The preparation of human epidermal skin membranes was carried out by heat separation (Kligman and Christophers, 1963) using full thickness abdominal cadaver skin. The skin was stored in a freezer at −20 °C until required and thawed for 1–2 h before preparation. To separate the epidermis, the full thickness skin was immersed and gently stirred in deionised water pre-heated to 60 °C for 45 s. After removal from the deionised water, the skin was put on a cork board with the dermal side down and the epidermal sheet was carefully peeled off by gently rubbing the surface of the skin with the gloved fingers. The separated skin was spread in a basin containing deionised water, mounted on filter paper (Whatman no. 1, UK) as a support and stored in aluminium foil at −20 °C until use. This method of storage is reported not to significantly affect the barrier properties of the skin for up to 466 days (Harrison et al., 1984).

2.6. Permeation studies using human skin

The permeation experiments were conducted at 32 (±1) °C using Franz-type diffusion cells as described in Section 2.3. The diffusional area was accurately measured for each Franz cell (~1.13 cm^2 diffusion area). Degassed PBS with 0.002% sodium azide was used as receptor phase. The heat separated epidermis was thawed and cut to appropriate size using scissors and assembled in the Franz cells with the filter paper support to help maintain skin integrity during the experiment. The possibility of drug binding and/or rate-limiting interference of the filter paper in the skin permeation was previously evaluated by comparing the permeation of the compound across silicone membranes with and without filter paper, which showed no differences. The skin was allowed to equilibrate with the receptor solution for 1 h before starting the experiment. Barrier integrity was assessed prior to beginning the experiment by measuring the impedance of the skin (Lawrence, 1997). Finite dose experiments were conducted by applying small volumes (8.9 $\mu L\,cm^{-2}$) of the test formulations (Table 1) at the skin surface using a micropipette. Contact between the pipette tip and the skin surface was minimised to avoid compromising the integrity of the membrane. Non-occluded donor and sink conditions were maintained throughout the experiment. Receptor phase samples were taken before applying the formulations ($t = 0$) to check for drug contamination in the receptor phase and analytical interference from material leaching from the skin. The concentration of methyl paraben in all samples was quantified using HPLC–UV (Section 2.8).

2.7. Mass balance

At the end of the finite dose permeation experiment, the *stratum corneum* surface of the skin was washed still mounted on the Franz cell with 1 mL of ethanol (99.7–100% (v/v) AnalaR® grade, BDH). After washing the donor compartment, the skin was removed from the filter paper support (considered to be part of the receptor compartment) and extracted three times sequentially using 1 mL of ethanol. The skin washes and extracts were centrifuged for 10 min at 12.7 (1000×) rpm/15.0 (1000×) rcf (Eppendorf centrifuge model 5415R) prior analysis. The concentration of methyl paraben in all samples was quantified using HPLC–UV (Section 2.8). The cumulative amount of methyl paraben permeated at the end of the experiment was used to calculate the recovery of the solute in the receptor compartment.

2.8. HPLC analysis

Methyl paraben quantification in all samples was carried out as previously described by Oliveira et al. (2011).

2.9. Data analysis

The permeation of methyl paraben was evaluated by plotting the cumulative amount permeated per unit surface area of the membrane ($mmol\,cm^{-2}$) against the collection time, as well as the fraction of the applied dose. The latter was estimated by dividing the cumulative amount permeated (in $\mu mol\,cm^{-2}$) by the dose applied (also in $\mu mol\,cm^{-2}$). The data treatment and statistics were performed using Microsoft® Office Excel 2003 and SPSS Statistics 17.0 Software. All results are presented as the mean ± standard deviation unless otherwise stated. Two-sample assuming equal variances Student's *t*-test (two tailed) and two-way ANOVA with replication using post hoc Tukey test have been performed to assess the statistical significance of the difference between means of different experiments (5% significance level, null hypothesis of equal means assuming normal distribution). The permeation parameters for the permeation across silicone membranes (diffusion coefficient, D in $cm^2\,min^{-1}$ and partition coefficient, K) and for the permeation across skin (partition parameter, P_1 in cm, and diffusion parameter, P_2 in h^{-1}) were obtained by fitting the data with Micromath Scientist® 3.0 for Windows, using appropriate Laplace transformation solutions to the diffusion equation which reflects the experimental conditions tested. The parameters P_1 ($P_1 = Kh$) and P_2 ($P_2 = D/h^2$) were estimated as representative of the vehicle–skin partition and diffusion coefficients because of the impossibility of knowing the exact path length (h) for drug diffusion across human skin. The finite dose model equations used in the fitting of the permeation data obtained with skin and model membranes were those already described by Santos et al. (2009) and Oliveira et al. (2010), respectively. Permeability coefficients (k_p in $cm\,min^{-1}$ or $cm\,h^{-1}$) and pseudo steady-state fluxes (J in $mmol\,cm^{-2}\,min^{-1}$ or $mmol\,cm^{-2}\,h^{-1}$) for the permeation of compounds across silicone membranes were calculated using the

Table 2
Solubility of methyl paraben (MP) in TC, DMI, IPM, EtOH and deionised water (di H_2O) at 32 °C (mean ± SD; $n = 3$) and respective solubility parameters for each solvent [van Krevelen and Hoftyzer type 3D solubility parameters estimated using Molecular Modelling Pro Demo software (version 6.2.3) according to the van Krevelen's group contribution method].

Solvent	Solubility parameter $(\text{cal cm}^{-3})^{1/2}$	MP solubility (mol L^{-1})
IPM	8.21	0.269 (±0.008)
DMI	9.97	2.038 (±0.052)
TC	10.62	2.843 (±0.027)
EtOH	12.26	2.422 (±0.105)
di H_2O	22.97	0.017 (±0.000)

values for K, D, P_1 and P_2 obtained from Scientist® following Eqs. (1) and (2), respectively. C_v is the concentration of the permeant in the donor solution and h is the diffusional path length, assumed to be the same as the model membrane thickness measured after the experiment (in cm).

$$k_p = \frac{KD}{h} = P_1 \times P_2 \quad (1)$$

$$J = k_p C_v \quad (2)$$

The lag times (t_{lag}) for drug permeation across silicone membranes and human skin were estimated using Eq. (3), respectively.

$$t_{lag} = \frac{h^2}{6D} = \frac{1}{6P_2} \quad (3)$$

3. Results and discussion

3.1. Solubility

The solubility of methyl paraben in each solvent, estimated at 32 °C, is shown in Table 2. The solubility of methyl paraben was highest in TC, followed by ethanol, DMI, IPM and water ($p < 0.05$). The results are in good agreement with the value reported in the literature for deionised water (Akomeah et al., 2004).

The solubility of a solid in vehicle (non-ideal solutions) can be described by Eq. (4), where X_2 is the molar fraction solubility; $\mathbf{\Phi}_1$ the volume fraction of solvent; V_2 the molar volume of the solute; R the gas constant; T the absolute temperature (Kelvin); T_0 the melting point of the solid; H_f the molar heat of fusion; and δ_1 and δ_2 the solubility parameters of the vehicle and the solute, respectively (Martin, 1993).

$$-\ln X_2 = \frac{\Delta H_f}{RT}\left(\frac{T_0 - T}{T_0}\right) + \frac{V_2 \Phi_1^2}{RT}(\delta_1 - \delta_2)^2 \quad (4)$$

These results are in agreement with the principles of regular solution theory embodied in Eq. (4), as the solvents with highest solubilisation capacity are those with solubility parameters which approach that of methyl paraben [i.e. 13.51 $(\text{cal cm}^{-3})^{1/2}$], thus minimising the last term of the equation: $(\delta_1 - \delta_2)^2$ (Vaughan, 1985; Hansen, 2000; Dias et al., 2007a).

3.2. Model membranes

3.2.1. IPM, DMI and TC

The permeation of methyl paraben from the saturated solution in IPM and from the IPM sat. + EtOH (50:50) formulation is shown in Fig. 2. The results show rapid permeation and subsequent drug depletion of methyl paraben from both IPM based formulations, with ~80% of the applied dose permeating after 90 min (Fig. 2A). In addition, Fig. 2B shows that both the saturated solution of methyl paraben in IPM and the formulation incorporating ethanol have equivalent efficiency in delivering the dose of methyl paraben applied to the membrane.

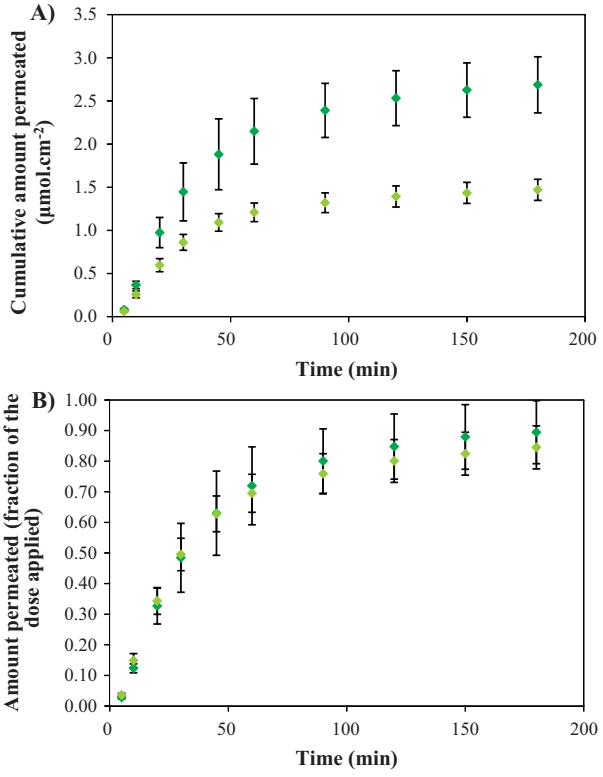

Fig. 2. Permeation of methyl paraben across silicone membrane as (A) cumulative amount and (B) fraction of the dose applied from (♦) saturated solutions in IPM and (♦) IPM sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C (mean ± SD; $n = 5$).

The dynamic interactions (e.g. solvent evaporation and uptake) of the applied IPM based formulations with the membrane are the likely cause of the early signs of drug depletion, as well as the limited information for lag time and (pseudo) steady-state permeation observed in Fig. 2A. Fitting the permeation data using non-linear software and a finite dose model (Section 2.9) resulted in poor correlation coefficients and a relatively high variance of the estimated permeation parameters. For this reason, the maximum flux values of methyl paraben across silicone membranes were investigated by direct differentiation of the cumulative amount permeated (Q

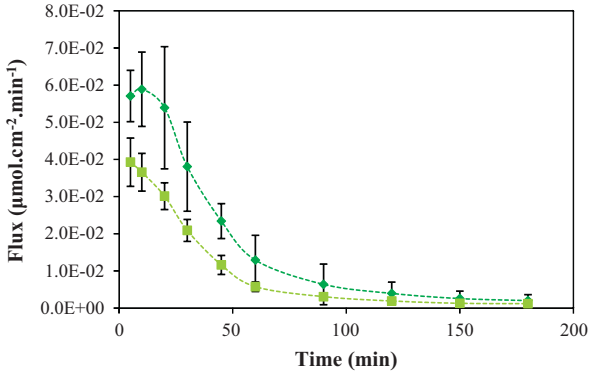

Fig. 3. Flux profile of methyl paraben across silicone membranes, estimated by differentiation of the permeation data in Fig. 2A with respect to time, from (♦) saturated solutions in IPM and (♦) IPM sat. + EtOH (50:50) formulations (finite dose) at 32 °C (mean ± SD; $n = 5$).

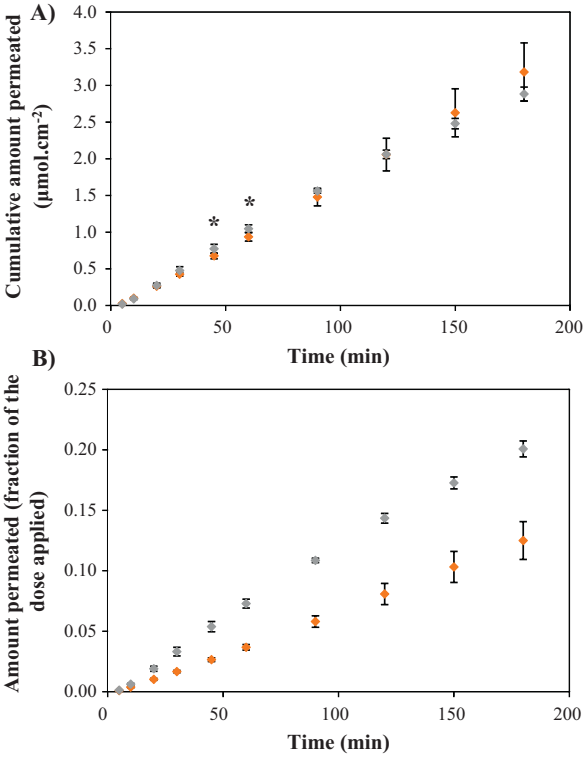

Fig. 4. Permeation of methyl paraben across silicone membrane as (A) cumulative amount and (B) fraction of the dose applied from (♦) saturated solutions in DMI and (◆) DMI sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C. The asterisk denotes significant differences between formulations (Student's *t*-test; $p < 0.05$) (mean ± SD; $n = 5$).

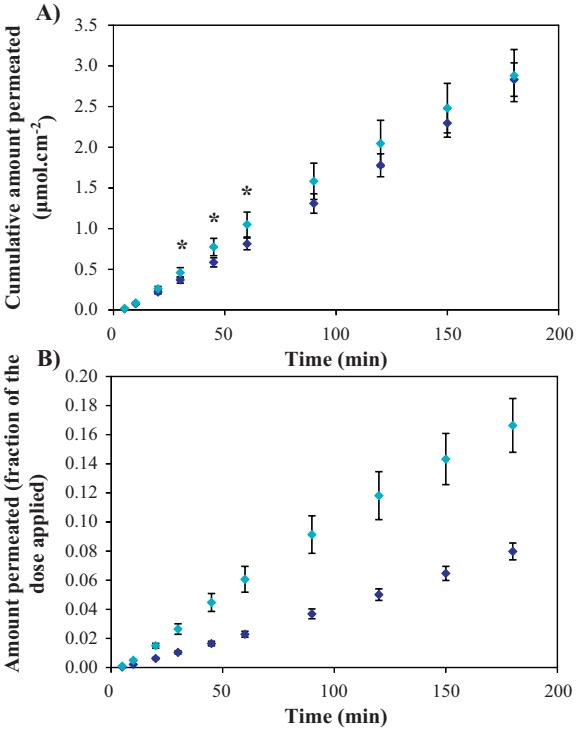

Fig. 5. Permeation of methyl paraben across silicone membrane as (A) cumulative amount and (B) fraction of the dose applied from (♦) saturated solutions in TC and (◆) TC sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C. The asterisk denotes significant differences between formulations (Student's *t*-test; $p < 0.05$) (mean ± SD; $n = 5$).

in µmol cm^{-2}) with respect to time (t in min), according to Fick's laws of diffusion (Eq. (5)):

$$J = \frac{DKC_v}{h} = \frac{dQ}{dt}. \quad (5)$$

Fig. 3 shows the plot of the change in the fluxes of methyl paraben across silicone membranes over time, estimated by differentiation of the permeation data obtained for both IPM based formulations (Fig. 2A) using Origin® 7.0 PRO software (OriginLab Corporation, US). The data in Fig. 3 show a slight initial increase in the methyl paraben flux, reaching a maximum value almost immediately after the start of the permeation. This is followed by an exponential-like decline towards zero flux, which is consistent with methyl paraben depletion from the formulations as it permeates through the silicone. The presence of ethanol in the formulation appears to have a moderate effect on the rate of permeation of methyl paraben across the silicone membrane (~1.5 fold difference between the average calculated maximum fluxes; $p < 0.05$).

Figs. 4 and 5 show the results obtained for the permeation of methyl paraben through silicone membranes from DMI and TC based formulations, respectively. The data show similar behaviour for the two vehicles: nearly identical permeation profiles were obtained for both the saturated solutions and the formulations incorporating ethanol (Figs. 4A and 5A). This implies similar permeation rates of methyl paraben across silicone membranes. Moreover, the data in Figs. 4B and 5B show that, although similar cumulative amounts of methyl paraben permeated over time, the formulations incorporating ethanol were more efficient in delivering the dose of methyl paraben applied compared with the respective saturated solution (~20% of the amount of methyl paraben applied permeated after 3 h, compared to ~13% for the saturated solution of methyl paraben in DMI; ~17% of the methyl paraben applied permeating after 3 h compared with only ~8% obtained for the saturated solution of methyl paraben in TC). The formulations containing ethanol were prepared by 1:1 dilution of the corresponding saturated solutions (Table 1), theoretically halving the thermodynamic activity of the solute of the saturated solutions. It would therefore be expected that the decreased driving force for drug permeation in these formulations should also result in comparatively lower fluxes of methyl paraben across the silicone membrane. The findings in Figs. 4 and 5 suggest that the ethanol readily evaporates from the small volume of the formulations applied at the surface of the silicone membrane (finite dose; non-occluded donor), leaving a saturated residue of methyl paraben in the vehicle. The evaporation of ethanol from the formulation means that the solute in the residual phase has the same thermodynamic activity as the corresponding saturated solution. Consequently, the same permeation rate of methyl paraben across the membrane is obtained for both DMI and TC based formulations. Similar findings have been reported by Tanaka et al. (1985) with silicone membranes while studying the influence of the evaporation of formulation components in the release of hydrocortisone butyrate propionate from oil-in-water cream and aqueous gel formulations. The authors compared drug release from several propylene glycol-based aqueous gels containing ethanol under occluded and non-occluded conditions, and also monitored the evaporation kinetics from the formulations. The observed differences in release rate were attributed to changes in drug solubility/thermodynamic activity in the residual formulation induced by the evaporation of ethanol and, subsequently, water from the formulation. More recently, Santos et al. (2011) have also described volatile solvent evaporation as an approach to attain supersaturated residual phases with varying degrees of saturation of fentanyl in propylene glycol. This resulted in increased permeation rates across silicone membranes, which

Table 3
Diffusion coefficient (D), vehicle–membrane partition coefficient (K), steady-state flux (J), permeability coefficient (k_p) and lag time (t_{lag}), obtained by non-linear modelling of the permeation data of methyl paraben across silicone membranes from each test formulation at 32 °C (n = 5).

	MP in DMI sat.	DMI sat. + EtOH (50:50)[a]	MP in TC sat.	TC sat. + EtOH (50:50)[a]
D ($cm^2\ min^{-1}$)	1.5E−05 (±9.1E−06)	3.1E−05 (±2.1E−05)	1.1E−05 (±3.3E−06)	4.0E−05 (±3.0E−05)
K	0.026 (±0.017)	0.012 (±0.008)	0.015 (±0.004)	0.007 (±0.004)
J ($\mu mol\ cm^{-2}\ min^{-1}$)	2.1E−02 (±3.6E−03)	2.0E−02 (±1.2E−03)	1.7E−02 (±1.4E−03)	1.9E−02 (±2.7E−03)
k_p ($cm\ min^{-1}$)	1.0E−05 (±1.8E−06)	9.9E−06 (±5.9E−07)	6.1E−06 (±5.0E−07)	6.7E−06 (±9.6E−07)
t_{lag} (min)	10.0 (±5.4)	5.2 (±3.2)	10.1 (±2.5)	4.3 (±2.4)

[a] Non-linear fitting of the data was conducted assuming a saturated residual phase at the surface of the membrane, after complete evaporation of the ethanol (finite dose model, using the saturated solubility as the initial concentration and considering 0.005 mL as the total volume applied).

were similar to equivalent formulations obtained by the co-solvent technique.

The permeation data in Figs. 4A and 5A was modelled using a finite dose model equation and non-linear modelling software (Section 2.9). The use of non-linear modelling software and appropriate analytical solutions to Fick's second law of diffusion allows the fitting of non-steady state diffusion profiles. It produces reliable partition and diffusion parameters with which to conduct a mechanistic evaluation of the effects of different formulations and formulation components in skin permeation. The permeation parameters obtained (i.e. flux, permeation coefficient, lag time, diffusion and partition coefficient) are shown in Table 3. The fitting of the permeation data from the DMI sat. + EtOH (50:50) and TC sat. + EtOH (50:50) formulations was conducted assuming complete evaporation of the volatile solvent from the formulation, leaving a saturated residue of the drug in the non-volatile vehicle (i.e. DMI and TC) at the surface of the membrane. These conditions were chosen following the studies of the loss of volatile solvent from the formulation (Section 3.3), indicating complete evaporation of the ethanol from the formulations about 6 min after application. The results in Table 3 confirm that the fluxes of methyl paraben across silicone membranes from both the saturated solution in DMI and the DMI sat. + EtOH (50:50) were the same within experimental error ($p > 0.05$). This was also the case for the TC based formulations.

However, comparable flux values were not observed for IPM based formulations (Fig. 2). IPM is a lipophilic solvent with high affinity for silicone membrane, as demonstrated by the relatively high sorption of this solvent into the membrane (Dias et al., 2007a). It is possible that the IPM from the formulations is rapidly taken up by the silicone membrane, possibly increasing methyl paraben partitioning (hence permeation; Fick's laws of diffusion: Eq. (5)) through a mechanism of action similar to that already observed (Oliveira et al., 2011). The fact that the maximum average flux of methyl paraben from the IPM + EtOH (50:50) formulation was slightly decreased compared with the saturated solution ($p < 0.05$) suggests that the permeation may be partially affected by the lowered solute activity. This is possibly the result of the presence of some ethanol in the formulation, regardless of the very fast evaporation kinetics (Section 3.3). The findings obtained with the IPM based formulations suggest that the presence of the volatile solvent has little effect on drug permeation, and that IPM is dominating the behaviour of the IPM sat. + EtOH (50:50) formulation.

It should also be noted from the results listed in Table 3 that the fitted permeation parameters K and D (and consequently t_{lag}) are very variable, particularly for the DMI and TC based formulations incorporating ethanol. Relatively smaller standard deviations are associated with the estimated fluxes and permeability coefficients, since the product of the two coefficients will likely compensate for the individual differences of K and D (Eq. (5)). The apparently poor fitting of the permeation data, especially for the formulations incorporating ethanol, may reflect their highly dynamic nature. In particular, the evaporation of ethanol and/or simultaneous transport of the vehicle into the membrane are likely to impact on the permeation of the solute across the membrane. The existence of more than one process occurring besides the permeation of methyl paraben across the membrane is not predicted by the finite dose model equation used. This will thus impact on the quality of the permeation parameters obtained by fitting the permeation data to these equations. In fact, a slight concave shape can be seen for the permeation of methyl paraben from both DMI and TC based formulations incorporating ethanol (Figs. 4A and 5A, respectively). This curvature appears in the permeation profiles despite the relatively small amount of solute permeated from the formulations (<20% of the dose applied), and therefore is not likely to be a typical response to significant donor depletion (Higuchi, 1962). Possible reasons for these observed trends may be as follows. (1) Significant vehicle depletion from the formulation may be occurring, promoting drug crystallisation in the formulation and decreasing its availability to permeate (Akhter and Barry, 1985; Chiang et al., 1989; Santos et al., 2010, 2011). Given the smaller volume of residual phase at the surface of the membrane, this effect should be more apparent in the formulations prepared with ethanol compared with the corresponding saturated solutions. (2) Ethanol has been reported as an effective permeation enhancer in silicone membranes, as well as in human skin (Alberti et al., 2001; Dias et al., 2007a,b; Watkinson et al., 2009). It is possible that, during its short residence time in the applied formulation, the ethanol may be taken up (i.e. interact) with the silicone membrane. This would lead to increased membrane concentrations of methyl paraben and thus contribute to increased flux in the initial stages of drug permeation. (3) Alternatively, it has been suggested in the literature that relatively fast permeation of ethanol from the formulation may create transient supersaturated states; thus leading to increased flux (Alberti et al., 2001; Dias et al., 2007b). A similar mechanism may also be occurring in the initial stages of methyl paraben permeation, because of the fast ethanol evaporation and depletion from the formulations.

3.2.2. EtOH and MP powder

To elucidate further the effect of the volatile solvent on drug permeation, the permeation of methyl paraben was also investigated from saturated solutions in ethanol, under finite dose conditions (non-occluded donor). Given the fast evaporation of the solvent and consequent depletion from the formulation (Section 3.3), the drug was expected to crystallise at the surface of the membrane, thus decreasing its availability to permeate. For this reason, the study was conducted over a 24 h period to facilitate the quantification of the methyl paraben permeated. Also because of significant solvent depletion from the formulation (evaporation), the mathematical equations derived to model non-steady state permeation from finite doses cannot be applied to the permeation data obtained using the saturated ethanol formulation. Therefore, the permeation rate was investigated by differentiation of the plot of cumulative amount permeated (in $\mu mol\ cm^{-2}$) with respect to time (in min), as previously described for IPM based formulations. Both permeation and flux profiles obtained for the permeation of methyl paraben from a finite dose of saturated solution in ethanol are shown in Fig. 6. The results show that the permeation of methyl paraben

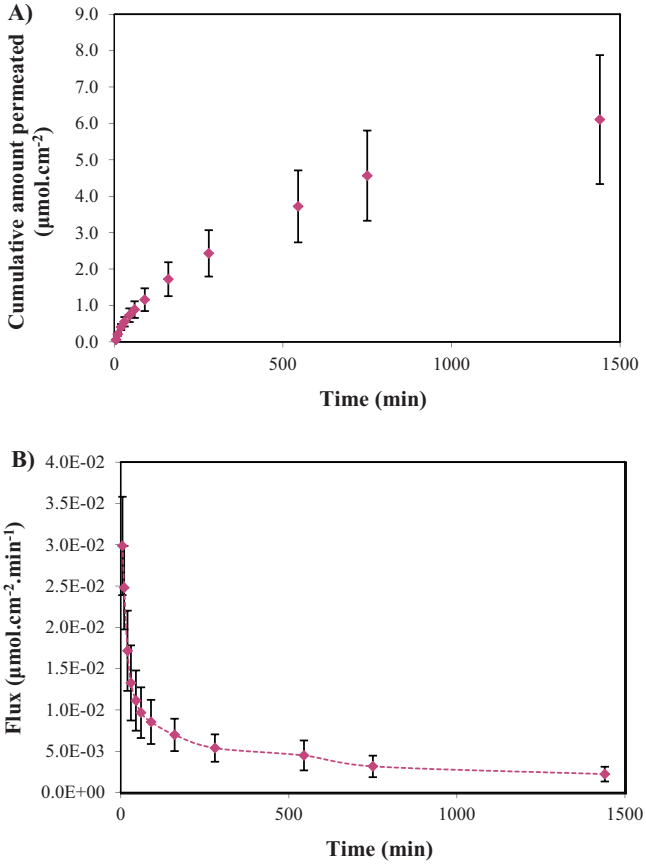

Fig. 6. Permeation of methyl paraben across silicone membrane from saturated solutions in EtOH, studied under finite dose conditions (non-occluded donor) at 32 °C: (A) cumulative amount permeated over 24 h; and (B) flux profile of methyl paraben estimated by differentiation of the cumulative amount permeated as a function of time (mean ± SD; $n = 5$).

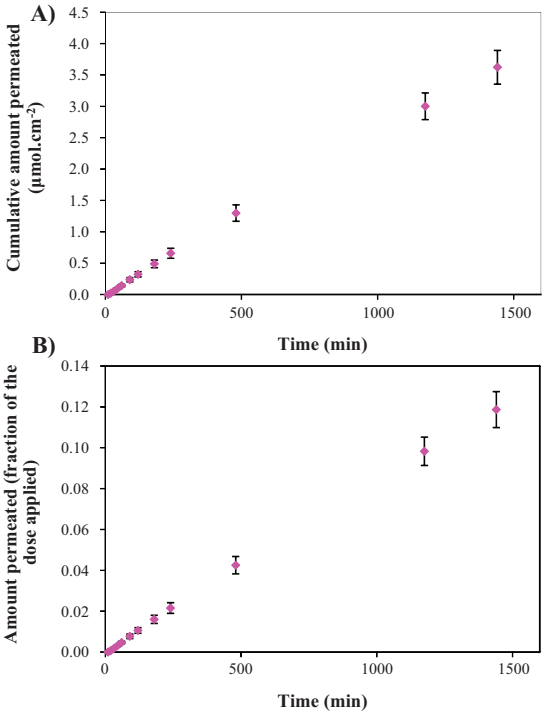

Fig. 7. Permeation of methyl paraben across silicone membrane as cumulative amount (A) and (B) fraction of the dose applied from the neat powder (~30.5 µmol cm^{-2}), studied under finite dose conditions (non-occluded donor) at 32 °C (mean ± SD; $n = 5$).

across silicone membranes from the saturated solution in ethanol was very low, with ~6% of the amount of methyl paraben applied in the formulation permeating after 160 min and only ~20% after 24 h. Additionally, there appears to be a non-steady state increase in the cumulative amount of methyl paraben permeated over time. The data in Fig. 6A show an initial period of relatively fast permeation (i.e. from 0 to 20 min). After 20–30 min there is a marked decrease in the permeation rate, followed by a steady decline until the end of the experiment (Fig. 6B). These findings are consistent with a scenario where an initial interaction of ethanol with the silicone membrane will promote a high initial concentration of the methyl paraben inside the membrane. This transient effect (supported by the ethanol evaporation studies; Section 3.3) is probably the cause for the observed initial increase in drug permeation. However, the fast depletion of ethanol from the applied formulation (evaporation and/or transport across the membrane) markedly decreases the residence time of the ethanol at the membrane surface, thus promoting crystallisation of the methyl paraben as a deposited film at the surface of the silicone membrane. In fact, crystals were already clearly visible at the surface of the membrane approx. 5 min after application. The crystallised drug will have limited ability to permeate through the membrane (Chiang et al., 1989; Santos et al., 2010, 2011), i.e. the process will be dissolution rate limited (Akhter and Barry, 1985), and so the permeation of methyl paraben is seen to decrease over time (Fig. 6A). Hence, the permeation of methyl paraben from saturated ethanol solutions applied at finite doses appears to be affected by a combination of two factors: (1) on the one hand there is the favourable interaction of ethanol with the silicone membrane, promoting an initially faster permeation. However, (2) fast depletion of the solvent from the donor (chiefly by evaporation) compromises its effect on the membrane and leads to drug crystallisation, thus decreasing the rate of permeation.

To assess the feasibility and extent of permeation from the drug crystals deposited at the surface of the membrane after solvent evaporation, the permeation of methyl paraben across silicone membranes from the neat powder was also investigated. A similar dose of methyl paraben to that applied in the previous study using the ethanol formulations was chosen for this investigation. The study was conducted over a 24 h period, and the donor compartment was occluded in an effort to prevent the accumulation of moisture from the atmosphere. Fig. 7 shows the results obtained for the permeation of methyl paraben through silicone membranes from the neat powder, both as the cumulative amount permeated (Fig. 7A) and in terms of the fraction of the dose applied (Fig. 7B). The results show that the permeation of methyl paraben in the absence of liquid solvent, albeit very small (~12% of the applied dose permeated after 24 h), was possible under the experimental conditions used. Also, contrary to what was previously reported for the ethanol formulations, steady-state permeation of methyl paraben across the silicone membrane can be seen throughout the time course of the experiment. The steady-state flux and lag time estimated directly by linear regression of the steady-state portion of the plot were 2.8E−03 (±2.8E−04) µmol cm^{-2} min^{-1} and 8.9 (±2.9) min, respectively. The latter is the same within experimental error ($p > 0.05$) as the lag time estimated, also by linear regression, for the permeation of methyl paraben under infinite dose conditions from water saturated solutions [i.e. 6.0 (±1.2) min, data not shown; non-interactive vehicle (Twist and Zatz, 1986)]. Romonchuk and Bunge (2006) investigated the permeation of 4-cyanophenol and methyl paraben from the neat powder and saturated aqueous solutions through both silicone membranes and human skin, to test the assumption that solid chemicals do not

permeate unless liquid is present. The study was conducted using vertical flow-through diffusion cells by adding about 0.1 g of the neat powder to the donor compartment for the first 1–3 h, after which it was replaced by a saturated aqueous solution of the drug and the experiment was continued for another 3 h (non-occluded donor). Under these conditions, the authors found that the flux through silicone membranes from the powder was not statistically different to that obtained from saturated aqueous solutions of methyl paraben, and only slightly smaller for 4-cyanophenol. In contrast, the results of the present study show that the flux obtained from the neat powder is considerably smaller than that estimated for the steady-state permeation of methyl paraben from the aqueous vehicle (i.e. 8.16E−03 ± 2.76E−04 μmol cm^{-2} min^{-1}; results not shown), even though both have equal thermodynamic activity (i.e. solute activity = 1). The exact reasons for this are not known. However it is possible that discrete contact of the powder with the silicone membrane, compared to the aqueous formulation, may have contributed to the differences observed. Additionally, the small amount of methyl paraben applied [~4.64 mg cm^{-2} vs. ~156.25 mg cm^{-2} used by Romonchuk and Bunge (2006)] may not have been sufficient to completely cover the surface diffusion area of the silicone membrane. In fact, a later study by the same research group (Ley and Bunge, 2007) has reported small but significant differences between the steady-state fluxes of methyl paraben and 4-cyanophenol from aqueous saturated solutions compared with the pure powder (~78.6 mg mg cm^{-2}) across silicone rubber membranes. The authors also found equal ratios between the fluxes from the aqueous solution and the powder for both chemicals, suggesting an underlying physical cause. Mathematical model simulations performed assuming non-volatile chemical sources covering limited fractions of the diffusional area of the membrane further supported the general explanation that, while diffusion proceeds through the entire membrane surface from a saturated solution, it does only from the membrane surface in direct contact with the powder.

Finally, the data in Fig. 6B show an initially high permeation of methyl paraben from the saturated solution in ethanol, which decreases exponentially over time and tends towards a minimum flux value, recorded at 24 h. Interestingly, the latter does not differ significantly ($p > 0.05$) from the flux estimated for the permeation of methyl paraben from the neat powder. These findings suggest that, as the initially high concentration of methyl paraben in the membrane (owing to the transient initial ethanol interaction) is depleted, permeation of methyl paraben will continue from the drug film deposited at the surface of the silicone membrane.

3.3. Kinetics of ethanol evaporation

The kinetics of evaporation of the volatile solvent from the TC, DMI and IPM based formulations incorporating ethanol was investigated. The study was conducted by monitoring the weight loss of the formulation over time at room temperature, after application of finite doses of the formulations at the surface of the silicone membrane. The results were compared with the evaporation rate from the neat volatile solvent, also applied to the silicone membrane. Controls on glass cover slips were also measured for 100% EtOH and the IPM sat. + EtOH (50:50) formulation, to assess the influence of possible vehicle interactions with the silicone membrane on the rate of ethanol evaporation from the formulations. Fig. 8 shows the results obtained for all formulations, presented in percentage of weight loss (wt.%) from the formulation applied at the beginning of the experiment. The results show a very fast evaporation of the ethanol from all the formulations: most of the volatile solvent evaporated ~6 min after application. This is in line with the findings for the finite dose studies (Section 3.1), showing similar fluxes of methyl paraben across silicone membranes

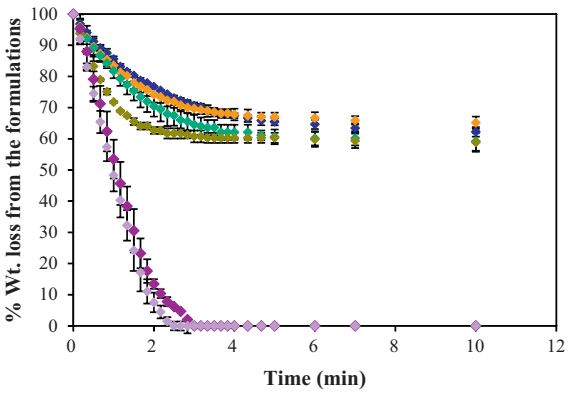

Fig. 8. Percent of weight loss (wt.%) from the formulations over time (solvent evaporation profiles). All formulations were applied on silicone membranes except otherwise specified: (♦) TC sat. + EtOH (50:50); (♦) DMI sat. + EtOH (50:50); (♦) IPM sat. + EtOH (50:50); (♦) IPM sat. + EtOH (50:50) applied on glass cover slip; (♦) 100% EtOH; and (♦) 100% EtOH applied on glass cover slip (mean ± SD; $n = 3$).

from DMI and TC based formulations incorporating ethanol, compared with the corresponding saturated solutions (Figs. 4A and 5A, respectively). In the TC, DMI and TC based formulations, the weight loss from the formulation corresponded to 70–80% of the volume of ethanol present, estimated using the density of the solvent at 25 °C (Wilhoit and Zwolinski, 1973). Also for the neat solvent, the ethanol recovery estimated from the weight loss of the formulation was ~80% of the volume of ethanol initially applied (10 μL). This was possibly because of evaporation of ethanol from the formulation occurring before the start of the experiment, owing to the equilibration of the balance. Additionally, Fig. 8 shows that the depletion of ethanol from the formulations applied in the silicone membrane was slightly faster from the IPM sat. + EtOH (50:50) formulation than from the TC and DMI based formulations. The reason for this could be the difference between the physicochemical properties of the solvents. TC and DMI are both hydrophilic solvents, and it is possible that increased affinity for ethanol (also hydrophilic) could be delaying its evaporation from the formulations. Conversely, the lipophilic character of IPM dictates its high affinity for silicone, consequently being taken up to a great extent by the membrane (Dias et al., 2007a). Upon application, the IPM sat. + EtOH (50:50) formulation is quickly taken up by the silicone membrane, possibly dragging some of the ethanol molecules inside the membrane and causing an additional delay in the evaporation of the volatile solvent. In fact, the comparison between the results obtained for the IPM sat. + EtOH (50:50) formulation applied to silicone membranes and to the glass cover slip (Fig. 8) shows that the evaporation of ethanol is faster for the latter. These results are in line with the hypothesis that some of the ethanol molecules are carried into the silicone membrane by the IPM. The evaporation of the trapped ethanol molecules would thus be rate-limited by their diffusion from the silicone matrix into the atmosphere. Finally, the data in Fig. 8 show very fast evaporation of ethanol when applied as neat solvent at the surface of the silicone membrane. The observed evaporation rate is very similar to that obtained from the neat solvent when applied in a glass cover slip. However, there appears to be a delay in the evaporation of ethanol, which is apparent when the neat ethanol is applied to the silicone membrane (Fig. 8: black arrow). The results suggest that, despite the short contact time, some of the ethanol molecules may be imbibed by the silicone membrane, thus delaying evaporation. These findings are consistent with the results from the finite dose study using saturated solutions of methyl paraben in ethanol (Section 3.2.2), suggesting an initial, transient interaction of the vehicle with the silicone membrane.

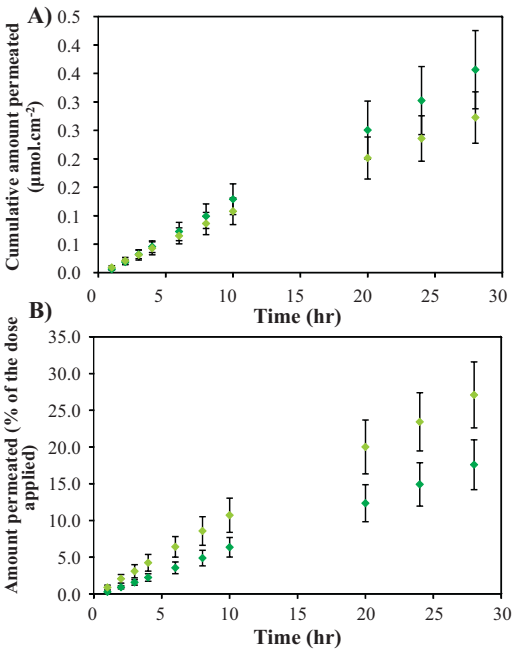

Fig. 9. Permeation of methyl paraben through human skin as (A) cumulative amount and (B) percent of the dose applied from (♦) saturated solutions in IPM and (♦) IPM sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C (mean ± SD; $n = 5$).

3.4. Human skin

The permeation data for methyl paraben in human skin from finite doses of the IPM, DMI and TC based formulations are shown in Figs. 9–11, respectively. The data from all vehicles show similar permeation rates for methyl paraben across human skin for both the saturated solution and the formulations incorporating the volatile solvent. The fitted partition and diffusion parameters, calculated lag times, permeation coefficients and fluxes obtained by non-linear modelling of the permeation data in Figs. 9A, 10A and 11A are listed in Table 4 for all the test formulations. The data show that the skin fluxes of methyl paraben across skin from both the saturated solutions and corresponding formulations incorporating ethanol were indeed the same within experimental error for IPM and DMI ($p > 0.05$). Although very similar profiles for cumulative amount permeated are observed for both TC based formulations (Fig. 11A), the methyl paraben flux from the TC sat. + EtOH (50:50) formulation was slightly higher ($p = 0.038$) than that from the saturated solution (Table 4). This was possibly an artefact of the modelling procedure, owing to the slightly convex (upwards) curvature that can be observed in the permeation profile for the formulation incorporating ethanol. Nevertheless, the findings for human skin are in overall agreement with those obtained using the model membranes, particularly for DMI and TC. This is consistent with a mechanism of action involving the ready evaporation of the ethanol from the formulation, leaving a saturated residual phase (i.e. solute activity = 1) of methyl paraben in the vehicle at the skin surface. Likewise, the presence of the volatile solvent in the formulations resulted in a marked increase of the dermal delivery efficiency of methyl paraben from all the vehicles tested (Figs. 9B, 10B and 11B).

Following the previous studies using silicone membranes, the permeation of methyl paraben through human skin was also studied from saturated solutions in EtOH under finite dose conditions (non-occluded donor). Fig. 12 shows the skin permeation data and respective flux profile, obtained by differentiation of the data in Fig. 12A with respect to time (Eq. (5)). The permeation profile of methyl paraben across the skin (Fig. 12A)

Table 4
Partition (P_1) and diffusion (P_2) parameters, pseudo steady-state flux (J), permeability coefficient (k_p) and lag time (t_{lag}), obtained by non-linear modelling of the permeation data of methyl paraben across human skin from each test formulation at 32 °C ($n = 5$).

	MP in IPM sat.	IPM sat. + EtOH (50:50)[a]	MP in DMI sat.	DMI sat. + EtOH (50:50)[a]	MP in TC sat.	TC sat. + EtOH (50:50)[a]
P_1 (cm)	2.92E−04 (±7.60E−05)	7.55E−05 (±8.51E−05)	1.33E−05 (±8.92E−06)	2.45E−05 (±7.73E−06)	4.62E−05 (±1.14E−05)	1.02E−04 (±5.48E−06)
P_2 (h^{-1})	0.225 (±0.063)	5.134 (±6.178)	0.491 (±0.501)	0.177 (±0.086)	0.099 (±0.023)	0.063 (±0.010)
J (µmol cm^{-2} h^{-1})	1.44E−02 (±3.03E−03)	1.14E−02 (±2.16E−03)	7.85E−03 (±1.75E−03)	7.98E−03 (±1.98E−03)	1.26E−02 (±2.36E−03)	1.64E−02 (±1.91E−03)
k_p (cm h^{-1})	6.32E−05 (±1.33E−05)	5.05E−05 (±9.53E−06)	3.65E−06 (±8.14E−07)	3.96E−06 (±9.83E−07)	4.44E−06 (±8.31E−07)	6.39E−06 (±7.47E−07)
t_{lag} (h)	0.8 (±0.2)	0.3 (±0.3)	0.6 (±0.4)	1.1 (±0.3)	1.7 (±0.3)	2.7 (±0.4)

[a] Non-linear fitting of the data was conducted assuming a saturated residual phase at the surface of the membrane, after complete evaporation of the ethanol (finite dose model, using the dose of methyl paraben applied in µmol cm^{-2} and considering an applied volume of 0.0044 mL cm^{-2}).

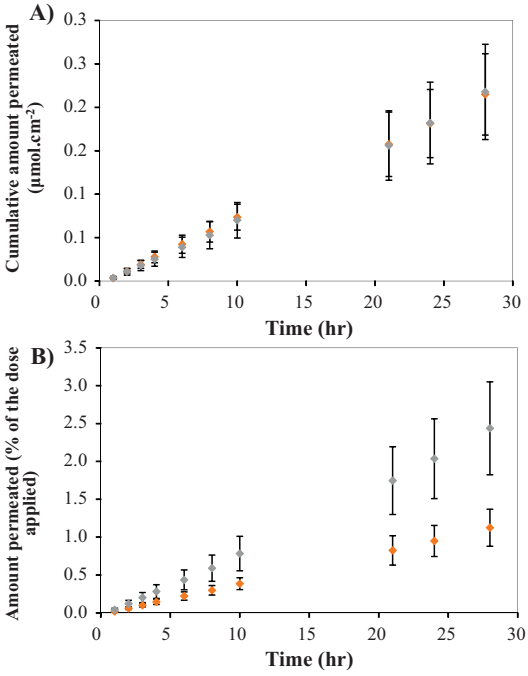

Fig. 10. Permeation of methyl paraben through human skin as (A) cumulative amount and (B) percent of the dose applied from (♦) saturated solutions in DMI and (♦) DMI sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C (mean ± SD; $4 \leq n \leq 5$).

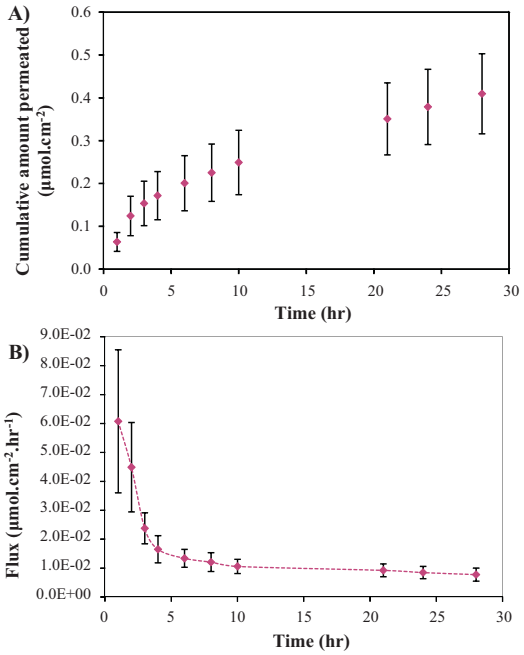

Fig. 12. (A) Cumulative amount of methyl paraben permeated through human skin from saturated solutions in EtOH, studied under finite dose conditions (non-occluded donor) at 32 °C and (B) flux profile obtained by differentiation of the permeation data with respect to time (mean ± SD; $n = 4$).

shows an initial period of relatively fast permeation (from 0 to 5 h), after which there is a marked decrease in the permeation rate until the end of the experiment. This was despite only a small fraction of the applied dose (i.e. ~2%) permeating over 28 h. Accordingly, Fig. 12B shows an initially high flux of methyl paraben across the skin from the saturated ethanol formulation (i.e. 6.1E−02 ± 2.5E−02 µmol cm^{-2} h^{-1}), decreasing exponentially over time until reaching a minimum at the end of the experiment. At this time, a deposited film of crystallised solute was clearly observed at the surface of the skin in the Franz cell. The lowest estimated skin flux for methyl paraben from saturated ethanol solutions was 7.8E−03 (±2.3E−03) µmol cm^{-2} h^{-1} (flux value at 28 h, Fig. 12B). This value is about one order of magnitude lower ($p < 0.05$) than that obtained for the permeation of methyl paraben from aqueous solutions, studied under infinite dose conditions (6.63E−02 ± 2.47E−03 µmol cm^{-2} h^{-1}; data not shown). The study conducted by Romonchuk and Bunge (2006) reported that, although the steady-state fluxes estimated for both compounds across silicone membranes from the neat powder and the aqueous solutions did not vary considerably, the observed fluxes in human skin were significantly reduced from the neat powder. The average skin fluxes reported by the authors for the neat powder relative to the saturated aqueous solutions were 7.24% and 9.02% for 4-cyanophenol and methyl paraben, respectively. In the present investigation, the skin flux of methyl paraben from the saturated ethanol solutions obtained after 28 h was ~11.8% of that estimated for saturated aqueous solutions under infinite dose conditions, which is consistent with the results of Romonchuk and Bunge (2006). These observations are also in line with the results obtained under the same conditions using silicone membranes (Section 3.2.2), suggesting that as the ethanol in the formulation

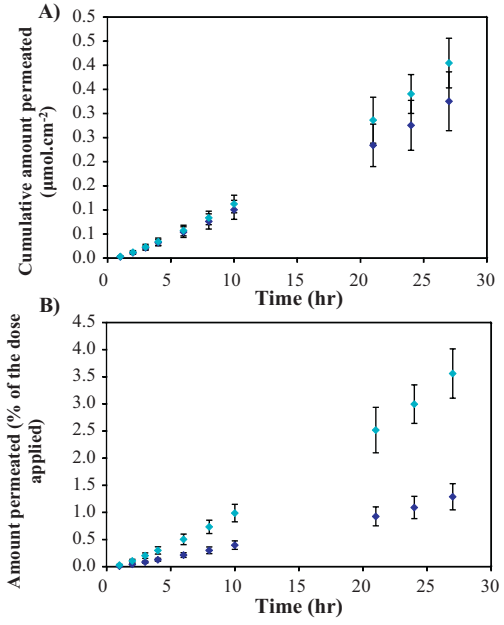

Fig. 11. Permeation of methyl paraben through human skin as (A) cumulative amount and (B) percent of the dose applied from (♦) saturated solutions in TC and (♦) TC sat. + EtOH (50:50) formulations, studied under finite dose conditions (non-occluded donor) at 32 °C (mean ± SD; $4 \leq n \leq 5$).

Table 5
Skin permeation of methyl paraben from each test formulation, studied under finite dose conditions (non-occluded donor) at 32 °C, expressed as cumulative amount (Q_{24}) and as percent of the dose applied (%Q_{24}) permeated after 24 h (mean ± SD; $4 \leq n \leq 5$).

Formulation	Q_{24} (µmol cm^{-2})	%Q_{24}
MP in IPM sat.	0.30 (±0.06)	14.92 (±2.95)
IPM sat. + EtOH (50:50)	0.24 (±0.04)	23.44 (±3.96)
MP in DMI sat.	0.18 (±0.04)	0.95 (±0.20)
DMI sat. + EtOH (50:50)	0.18 (±0.05)	2.04 (±0.53)
MP in TC sat.	0.28 (±0.05)	1.09 (±0.20)
TC sat. + EtOH (50:50)	0.34 (±0.04)	3.00 (±0.35)
MP in EtOH sat.	0.38 (±0.09)	1.81 (±0.42)

Table 6
Statistical analysis of the Q_{24} data using one-way ANOVA with replication (post hoc Tukey test; 5% significance level).

	IPM sat. + EtOH (50:50)	MP in DMI sat.	DMI sat. + EtOH (50:50)	MP in TC sat.	TC sat. + EtOH (50:50)	MP in EtOH sat.
MP in IPM sat.		*	*			
	IPM sat. + EtOH (50:50)				*	*
		MP in DMI sat.			*	*
			DMI sat. + EtOH (50:50)		*	*
				MP in TC sat.		
					TC sat. + EtOH (50:50)	

The asterisk denotes significant differences between formulations ($p < 0.05$)

is depleted the drug film deposited at the surface of the skin has limited dermal absorption. The overall findings point towards an interaction of ethanol with the skin, promoting an initially high concentration of methyl paraben in the SC and leading to the observed initial increase in permeation. However, the enhancing effect of this vehicle is limited by its extremely short residence time in the formulation, owing to its fast evaporation. This causes most of the dose of methyl paraben applied to crystallise as a deposited film at the skin surface (observed experimentally), and probably also in the skin; thus decreasing its availability to permeate. This is confirmed by the mass balance study conducted at the end of the experiment, showing the majority of the methyl paraben applied in the ethanol saturated formulation (~94%) remaining at the surface of the skin after 28 h of application (Fig. 13). A similar mechanism of action has been reported by Akhter and Barry (1985) for the permeation of ibuprofen and flurbiprofen from drug films deposited by acetone evaporation on human skin in vitro. Their results showed an initial ibuprofen and flurbiprofen permeation rate maximum, owing to drug partitioning from the acetone solution into the skin and subsequent permeation. After this, the permeation rates decreased for the drug films deposited at the skin surface, following acetone evaporation. Stinchcomb et al. (1999) have also investigated the skin uptake of 4-cyanophenol in vivo from volatile and non-volatile solvents. The authors found a 2 to 8-fold increase in the amount of chemical taken up by the SC when applied in an acetone solution compared with water saturated solutions, even though a longer exposure time was used in the latter. Stinchcomb et al. (1999) also proposed that most of the solute was taken up while the vehicle was present, and that little additional uptake seemed to take place after its complete evaporation.

Finally, all the formulations tested were assessed for their ability to deliver methyl paraben through the skin when applied at clinically relevant doses (finite dose). Table 5 summarises the cumulative amounts of methyl paraben permeated after 24 h (Q_{24}) from each test formulation, used as a measure of the extent of skin permeation; also represented in terms of the percentage of the dose applied permeated after that period (Q_{24}%). The data show similarly high Q_{24} from IPM and TC based formulations and also the ethanol saturated solution, followed by the DMI based formulations. Table 6 lists the results of the statistical analysis of variance (ANOVA) performed on the Q_{24} data. Surprisingly, a relatively high amount of methyl paraben was delivered through the skin from the saturated ethanol solution, despite the short residence time of the vehicle at the surface of the skin (Table 5). The $\%Q_{24}$ data show clear differences in the efficiency of the formulations tested to deliver the dose of methyl paraben applied. The highest value was observed using the IPM sat. + EtOH (50:50) formulation, followed by the saturated IPM solution and the TC sat. + EtOH (50:50) formulation ($p < 0.05$). Both the DMI sat. + EtOH (50:50) formulation and the ethanol saturated solution delivered about 2% of the dose applied after 24 h ($p > 0.05$), whereas the least effective formulations were the saturated solutions in both TC and DMI (~1% of the methyl paraben permeated after 24 h; $p > 0.05$).

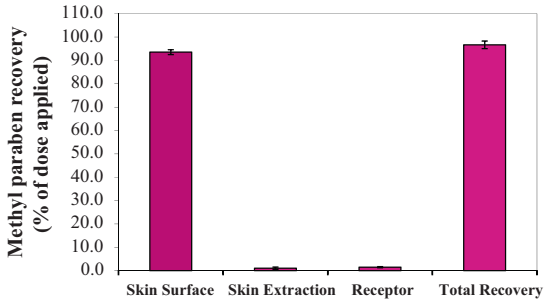

Fig. 13. Total recovery of methyl paraben in each compartment (skin surface, skin and receptor compartment) obtained after finite dose studies using the EtOH saturated solution (mean ± SD; $n = 4$).

4. Conclusions

The presence of EtOH in the IPM, DMI and TC formulations tested had little effect on the flux of methyl paraben through the skin. The results obtained in human skin are in good agreement with the findings from the silicone membranes. This suggests that ready evaporation of EtOH from the formulations applied at the skin surface occurs, leaving a saturated residue of the drug in the vehicle. The fluxes from formulations incorporating volatile solvent were, hence, similar to those from the respective saturated solutions, and the dermal delivery efficiency of the formulation was markedly increased. This is further substantiated by the findings of the study of the kinetics of EtOH evaporation, showing complete ethanol evaporation from the formulations applied at the surface of the membrane in less than 6 min.

The skin permeation of methyl paraben from saturated ethanol solutions applied at finite doses appears to be affected by a combination of two factors. Firstly, the favourable interaction of ethanol with the skin promotes an initially faster permeation. However, fast solvent depletion from the donor (chiefly by evaporation) limits its enhancing effect, leading to methyl paraben crystallisation. Drug crystallisation on or in the skin will have a direct impact on both the rate and extent of permeation. Nevertheless, a significant amount of MP was delivered through the skin from the saturated solution in EtOH, despite its short residence time at the skin surface. This may have important implications in terms of exposure to such formulations and potential toxicological effects.

The reported findings demonstrate the potential of volatile solvents to optimise the efficiency of drug delivery to the skin. As demonstrated in this work, these can be used to decrease the drug loading in formulations, which is likely to be advantageous from both pharmaceutical and regulatory perspectives.

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