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Formulation issues associated with transdermal fentanyl delivery

P. Santos [a], A.C. Watkinson [b], J. Hadgraft [a], M.E. Lane [a,*]

[a] Department of Pharmaceutics, The School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, United Kingdom
[b] Acrux Limited, 103-113 Stanley Street, West Melbourne, VIC 3003, Australia

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ABSTRACT

Supersaturation has previously been studied as a mechanism to enhance membrane transport of fentanyl from propylene glycol:water formulations (PG:H_2O) across silicone. In this study these supersaturated fentanyl formulations were evaluated in human skin. A number of polymers were also screened for their ability to stabilise the supersaturated formulations and permeation was evaluated for both infinite and finite doses. For infinite dose studies, permeation in skin increased linearly with increasing degree of drug saturation (DS) for formulations containing 0.5, 1, 2 DS of fentanyl and a 3 DS formulation stabilised with 1% (w/v) hydroxypropylcellulose (HPC). An excellent correlation was obtained for flux values in silicone compared with flux values in skin, for infinite dose studies for formulations containing 0.5, 1, 2 DS of fentanyl and the 3 DS formulation stabilised HPC. The concentration of the fentanyl in the stratum corneum also increased in proportion to the DS. However the same trend was not observed for finite dose studies. This is because the depletion of the solvent carrier promotes drug crystallisation with consequent implications for membrane transport. Tape-stripping experiments indicated that supersaturation of the drug is maintained in the outer layers of the stratum corneum. The ideal vehicle must, therefore, maintain the drug in solution on and in the skin in a sustained manner for effective transdermal delivery.
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1. Introduction

Supersaturation has been explored as a strategy to enhance the skin permeation of drugs by many researchers (Davis and Hadgraft, 1991; Pellett et al., 1994; Moser et al., 2001; Santos et al., 2009). Maximum thermodynamic activity in solution is achieved when the drug exists at saturation. However, it is possible to employ techniques which create supersaturated states by increasing the concentration of the solubilised drug to values higher than its solubility in the formulation (Hadgraft, 2004). Supersaturated systems are by nature, thermodynamically unstable and drug crystallisation in the formulation will tend to occur over time, decreasing the degree of saturation (Santos et al., 2010). Consequently, the stability of supersaturated formulations must be improved by the addition of anti-nucleant and/or anti-crystal growth agents, such as polymers (Raghavan et al., 2000).

We have previously examined the permeation of supersaturated formulations of fentanyl in propylene glycol:water (PG/H_2O) across silicone membrane (Santos et al., 2011). Silicone was chosen in order to separate the effects of supersaturation from other possible influences of formulation components on biological membranes. Although good correlations between the degree of saturation (DS) of the drug in the formulation and the flux were obtained the observed permeation was lower than predicted. This decrease was attributed to drug crystallisation. The objectives of the present work were (1) To examine the behaviour of these formulations using human skin; (2) To screen a range of polymers screened for their ability to stabilise (prevent crystallisation) the formulations and (3) To compare both infinite and finite doses in order to determine how vehicle depletion from small applied volumes may affect permeation.

2. Materials and methods

2.1. Materials

Fentanyl free base (racemic mixture) was a gift from Acrux, Ltd. (Melbourne, Australia). Acetonitrile, methanol and water were HPLC grade. Propylene glycol (PG), 1-heptanes sulphonic acid, hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), polyvinylpyrrolidone K90 (PVP K90) and 1-heptane sulphonic acid were purchased from Sigma (Melbourne, Australia). Scotch tape (3 M, Australia) was used in tape stripping. Deionised water was used to prepare all formulations. Human (female) abdominal tissue obtained after plastic surgery, with informed consent and appropriate ethical approval was stored in poly-

* Corresponding author. Tel.: +44 207 7535821; fax: +44 870 1659275.
E-mail address: majella.lane@btinternet.com (M.E. Lane).

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thene bags at −20 °C until required. For diffusion studies, prepared dermatomed skin (0.2 mm thick) was defrosted at room temperature for approximately 1–2 h before use. One single donor was used to minimise the variation associated with different donors.

2.2. Preparation and stability of supersaturated solutions

Supersaturation was produced using the cosolvent method as reported previously (Santos et al., 2011). For stability studies, PG/H_2O formulations (60:40, v/v) with 0.5, 1, 2, 3 and 5 DS were prepared where 1 DS indicates a formulation containing fentanyl at its saturated solubility, 2 DS indicates a formulation containing fentanyl at twice its saturated solubility etc. 1 ml of these solutions was added to Eppendorf® tubes and stored at 32 °C. At pre-determined intervals, these tubes were centrifuged and the remaining drug in solution was quantified.

2.3. Polymer screening

A preliminary study of the effect of polymers (HPC, HPMC, and PVP K90) on the stabilisation of a supersaturated formulation was performed by visual inspection. An aliquot (5 μl) of the supersaturated solution obtained from a PG:H_2O (60:40, v/v) with 5DS of fentanyl and with a final polymer concentration of 1% (w/v) was spread on a glass slide. The effect of polymers was evaluated by assessing the crystal growth rate by polarized light microscopy (Olympus SZX12 Microscope, Olympus instruments, Japan). Images were acquired using Insight QE Camera Model 4.2 (Diagnostic Instruments Inc., United States).

2.4. Permeation studies

In vitro skin diffusion studies were performed using stainless steel flow-through diffusion cells (Acrux, Australia) with an area available for drug diffusion of 1 cm^2. Isotonic phosphate buffer pH 7.4 (preserved with 0.1% (w/v) sodium azide) was used as the receptor solution. Infinite (200 μl/cm^2) and finite (5 μl/cm^2) volumes of donor solution were applied across the skin using a micropipette and spread with the pipette tip. The diffusion samples were collected every 1–4 h over a 24 h period using an automated fraction collector (ISCO Retriever II, Netherlands). At 24 h diffusion, the diffusion cells were dismantled and the skin was tape-stripped. Complete removal of the SC was indicated by the appearance of a white and shiny surface, characteristic of the viable epidermis. The weight of the skin was measured after the first, second and the last tape strip, and the amount removed by each tape was calculated by weight difference. The drug was extracted twice using 1 ml of methanol and analysed by HPLC after centrifugation and appropriate dilution with methanol.

2.5. HPLC analysis

Fentanyl concentrations were determined using reverse phase High-Performance Liquid Chromatography (HPLC) in conjunction with UV detection as reported previously (Santos et al., 2011). Calibration curves for each assay were constructed using standard fentanyl solutions at concentrations within the range of 0.05–10 μg/ml. A linear relationship between peak area and concentration was confirmed by the correlation coefficient generated by linear regression (using a least squares method) of the calibration curve. The linearity of each assay was higher than 0.995, and the accuracy between 95 and 105%.

2.6. Data analysis

2.6.1. Flux and ER

Scientist® Version 3.0 (Micromath Inc., Salt Lake City, UT, United States) was used to fit diffusion equations to experimental data. The finite dose model expressed as a Laplace transforms was used (Eq. (1)):

$$\overline{\text{Amount}} = \frac{AP_1Q_0}{s\left[V\sqrt{\frac{s}{P_2}}\sinh\sqrt{\frac{s}{P_2}} + P_1A\cosh\sqrt{\frac{s}{P_2}}\right]} \quad (1)$$

where s is the Laplace variable, P_1 and P_2, also known as apparent partition and apparent diffusion coefficients are defined as follows:

$$P_1 = Kh \quad (2)$$

$$P_2 = \frac{D}{h^2} \quad (3)$$

K and D are the partition and diffusion coefficients, h is the thickness and A is the area of the membrane, V is the volume of donor phase, and Q_0 is the amount applied. Steady state flux (J_{ss}) was calculated using Eq. (4).

$$J_{ss} = P_1P_2AC_L \quad (4)$$

where C_L is the drug concentration in the donor phase. The enhancement ratio (ER) is defined as the ratio between the flux of the supersaturated formulation under study and the flux of the saturated formulations. The permeability coefficient (k_p) was obtained by the product of $P_1 \times P_2$.

2.6.2. Statistical analysis

Statistical significance was determined using one-way analysis of variance (ANOVA). Post hoc all pair-wise multiple comparison of the means within different groups was performed using the Post hoc Bonferroni Test. A probability of $p < 0.05$ was considered statistically significant. 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 ± SD, unless otherwise stated.

3. Results and discussion

3.1. Polymer screening

The influence of polymers (HPC, HPMC, PVP K90) on stabilisation of supersaturated formulations was investigated by visual inspection. Spontaneous nucleation was observed for the PG/H_2O formulation prepared with 5 DS when no polymer was used (Fig. 1). In the presence of 1% (w/v) of HPC the crystallisation of the 5 DS formulation was retarded but not inhibited. HPMC or PVP K90 at 1% (w/v) showed only slight retardation on the crystallisation of fentanyl for the 5 DS formulation compared with HPC. No significant changes were observed in the crystal habit for any of the polymers studied. Typically, crystals exhibited a long needle-shaped morphology and were agglomerated in star shape bundles.

In the absence of polymer, the formulation with fentanyl at 3 DS started to crystallise after 120 min. However, after adding 1% HPC (w/v), the crystallisation was inhibited for 24 h. The 2 DS formulation without polymer remained stable over 24 h. Although polymer concentrations up to 5% (w/v) were investigated (data not shown) they failed to stabilise the 5 DS formulation, as crystallisation was immediate.

3.2. Infinite dose studies

The potential use of the supersaturated formulations for topical and transdermal drug delivery was further evaluated *in vitro* using

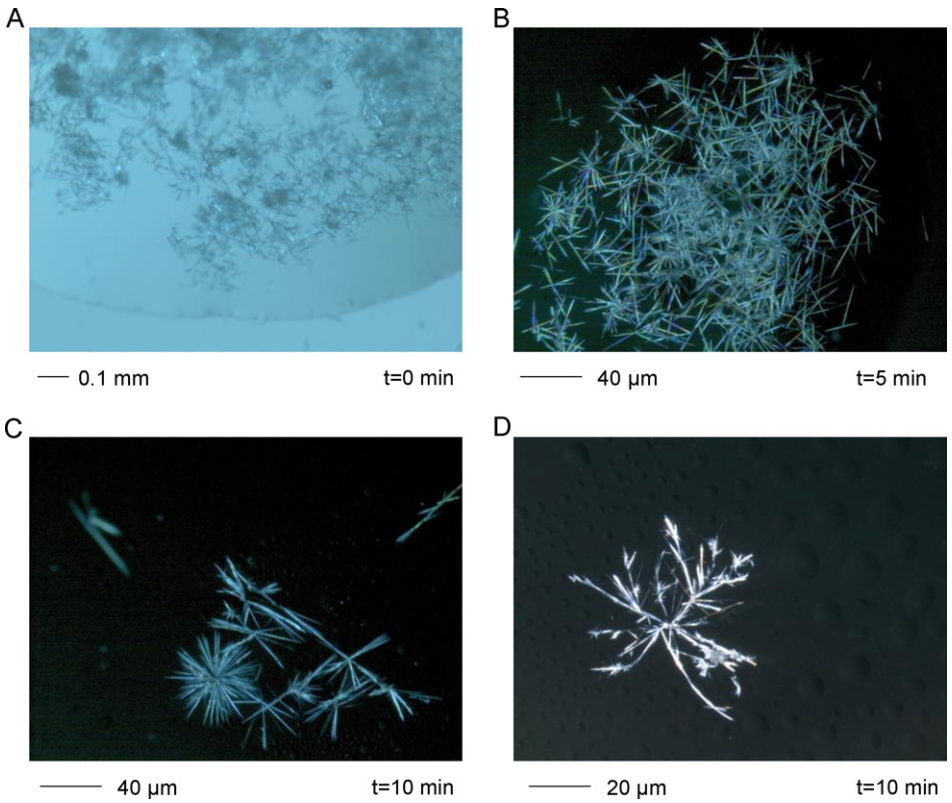

Fig. 1. Microscopic visualisation of crystal formation and growth in PG:water (60:40, v/v) containing 5DS of fentanyl, with and without additives: A – no additive (magnification 4×); B – 1% HPMC (magnification 20×); C – 1% PVP K90 (magnification 10×) and D – 1% HPC (magnification 36×).

abdominal human skin. Fig. 2 shows the cumulative amount of drug permeated through human skin after application of 200 µl/cm^2 of formulations with 0.5, 1, 2, 3 DS of fentanyl, and 3 DS of fentanyl (stabilised with 1% HPC, w/v) over 24 h.

The permeation of fentanyl across the skin increases with increasing DS of the formulation, confirming that supersaturated formulations enhance drug permeation and in line with previous *in vitro* silicone studies and reports from the literature (Davis and Hadgraft, 1991, 1993; Pellett et al., 1994; Iervolino et al., 2000; Hou and Siegel, 2006; Santos et al., 2010, 2011).

Formulations with 3 DS without polymer produced the same permeation profile as the 1 DS formulation, which reflects the instability of the 3 DS formulation. After 120 min, all the drug had crystallised in this formulation (data not shown) thus resulting in the same driving force as the 1 DS formulation. However, the presence of 1% (w/v) of anti-nucleant agent (HPC) stabilised the 3 DS supersaturated formulation over 24 h, resulting in a three-fold increase in drug flux compared with the 1 DS formulation.

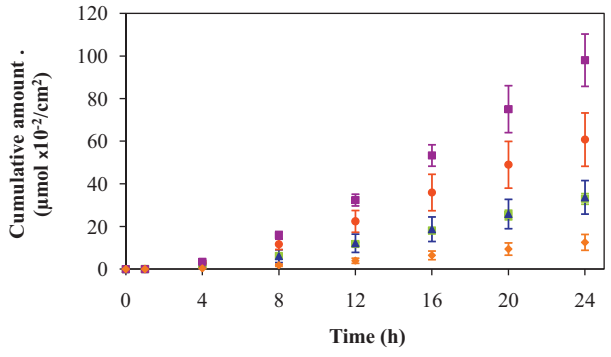

Fig. 2. Fentanyl permeation across skin from PG/H_2O (60:40, v/v) with (♦) 0.5 DS, (▲) 1 DS, (●) 2 DS, (■) 3 DS fentanyl without polymer, and (■) 3 DS fentanyl with 1% HPC, under infinite dose conditions. Each data point represents the mean ± SD (n = 4–8).

The values of membrane permeability (k_p), apparent diffusion (P_2) and apparent partition coefficients (P_1), obtained by fitting experimental data using an infinite diffusion model are shown in Table 1. The steady state flux, lag time and ER for infinite dose studies at different DS are also reported. No significant differences were seen in the t_{lag}, P_1, P_2, or k_p between formulations. This implies that the increased flux was exclusively the result of increased thermodynamic activity of the drug.

The concentration of fentanyl recovered from the SC increased proportionally with the DS ($p < 0.05$, ANOVA, Fig. 3). The concentration in the SC following application of 2 and 3 DS with 1% (w/v) HDC, is 1.6 and 3.3 times greater than the amount recovered from a saturated solution ($p < 0.05$, ANOVA). Similarly, the fentanyl concentration in the SC resulting from the application of subsaturated formulations is approximately half (2.2 ± 0.4 µmol/g of SC/cm^2) of the SC concentration following application of a saturated solution (3.9 ± 0.3 µmol/g of SC/cm^2). These extraction results are in agreement with the flux ER (Table 1) for the same formulations. Additionally, the 3DS formulation without polymer shows a similar concentration in the skin (2.6 ± 0.7 µmol/g of SC/cm^2) to that observed for the 1DS formulation.

The first two tape strips were also quantified in order to confirm that the first layers of the SC are able to maintain supersaturation states. The results obtained from the first tape strip were discarded because of the high variability associated with the washing procedure. However, the results from the 2nd tape strip were less variable (Fig. 3). The concentration recovered in the SC removed by the 2nd tape is 1.7–2.1-fold higher than the respective con-

Table 1
Permeation fluxes, P_1, P_2, lag times for infinite dose studies. Steady state flux (J_{ss}) was estimated using Eq. (4) and the values of k_p obtained by fitting. The flux ER is the ratio between the mean steady state flux and the 1 DS mean flux. Each data point represents the mean ± SD (n = 4–8).

Formulation	J_{ss} (μmol × 10^{-2}/cm^2/h)	t_{lag} (h)	P_1 (cm)	P_2 (h^{-1})	k_p (×10^{-4} cm/h)	ER
0.5 (subsaturated)	0.7 ± 0.2	7.2 ± 0.9	0.029 ± 0.010	0.023 ± 0.003	6.7 ± 2.0	~0.4
1 DS (saturated)	1.7 ± 0.3	6.2 ± 1.4	0.030 ± 0.011	0.028 ± 0.007	7.9 ± 1.5	1
2 DS	4.0 ± 0.5	5.0 ± 2.3	0.026 ± 0.011	0.039 ± 0.015	9.0 ± 1.2	~2
3 DS	1.6 ± 0.3	5.2 ± 0.8	0.023 ± 0.007	0.033 ± 0.005	7.4 ± 1.3	1
3 DS with 1% w/v HPC	5.4 ± 0.1	6.5 ± 0.7	0.032 ± 0.003	0.026 ± 0.003	8.2 ± 0.9	~3

centration from the rest of the SC, which agrees with the linear concentration gradient expected inside the skin at steady state. These results demonstrate that the first layers of the SC are indeed capable of supporting supersaturated states.

The layers of SC are composed of corneocytes surrounded by a lipid environment. It is generally accepted that most of the molecules diffuse across the SC through this lipid pathway and around the corneocytes. Therefore, it is plausible that the ability of the first layers of the SC to maintain supersaturated states is somehow related to the anti-nucleant properties of this unique lipid combination, as suggested by Pellett et al. (1997).

The relationship between the steady state fluxes through the skin and the concentration of fentanyl in the skin is shown in Fig. 4. The permeation of fentanyl through skin increased proportionally with uptake of the drug into the SC (r^2 = 0.87). This finding is in agreement with published data for the skin permeation of piroxicam and a lavendustin derivative (Pellett et al., 1997; Moser et al., 2001). Megrab et al. (1995) have also shown that the membrane (silicone and human epidermis) uptake ratio of oestradiol (the ratio between the membrane uptake from a supersaturated and saturated solution) was proportional to the DS of the formulation.

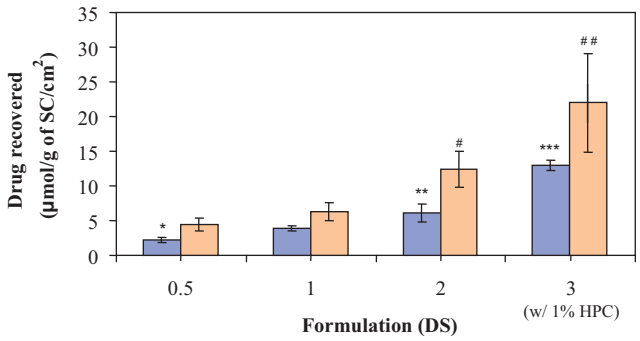

Fig. 3. Fentanyl concentration in the (■) SC and in the (■) 2nd tape strip after application of 200 μl/cm^2 of PG/H_2O (60:40, v/v) containing 0.5, 1, 2 DS fentanyl and 3 DS fentanyl (stabilised with 1% HPC, w/v). Each bar represents the mean ± SD, n = 4–8. $^{*}p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.005$, drug recovered in the SC is statistically different from 1 DS formulations. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$, drug recovered in the 2nd tape strip is statistically different from 1 DS formulations (ANOVA, Post hoc Bonferroni Test).

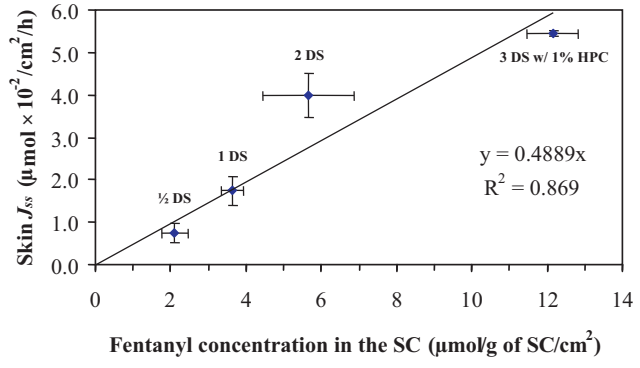

Fig. 4. The fentanyl concentration in the SC as a function of the steady state flux achieved by supersaturated formulations with 0.5, 1, 2 DS fentanyl, and 3 DS fentanyl (stabilised with 1% HPC, w/v), in PG:H_2O (60:40, v/v). Each data point represents the mean ± SD (n = 4–8).

3.3. *Infinite dose studies: comparison of flux through silicone membrane and skin*

Flux data for the formulations across silicone has been reported in a previous paper (Santos et al., 2011). The relationship between the flux of fentanyl through silicone and skin, infinite dose studies with subsaturated, saturated and supersaturated formulations stable for 24 h is shown in Fig. 5. There is a very good correlation (r^2 = 0.97) between the flux of fentanyl through silicone membranes and the skin, indicating that the thermodynamic activity is the main factor controlling the drug permeation in both membranes. Similar results were earlier reported for ibuprofen (Iervolino et al., 2001) and salicylic acid (Leveque et al., 2006).

The flux of fentanyl across silicone is more than three times higher than the flux of fentanyl through human skin. This is because of the differences in the diffusion pathlength and diffusion coefficients in the two membranes: Additionally, there are significant structural differences between simple synthetic membranes and the complexity found in biological membranes, thus resulting in different drug diffusivities in the membrane (Nacht and Yeung, 1985). Assuming a diffusion pathlength in skin of 350 μm (Albery and Hadgraft, 1979) and using the values described in Table 1 for skin and previous reported values for silicone (Santos et al., 2011) the drug diffusivity in silicone is calculated to be one order of magnitude higher than the diffusivity in the skin.

3.4. *Finite dose studies*

In vitro finite dose studies (5 μl/cm^2) with human skin were also conducted with all formulations to simulate better typical clinical usage (Fig. 6). Saturated and supersaturated formulations produce similar fluxes, for both formulations. In addition, the use of 1%

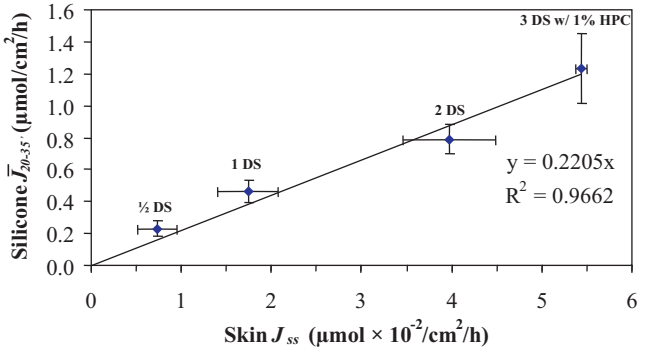

Fig. 5. Fentanyl permeation fluxes achieved with subsaturated (0.5 DS), saturated and supersaturated solutions (2 and 3 DS with 1% HPC, w/v), composed of PG:H_2O (60:40, v/v), through silicone and skin membranes, when applied as an infinite dose. Each data point represent the mean ± SD (n = 3–8).

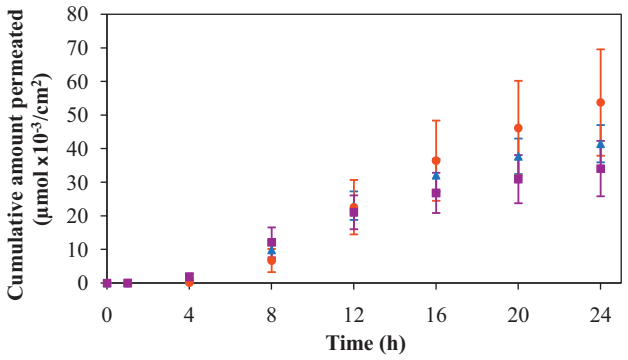

Fig. 6. Fentanyl permeation across dermatomed skin from PG/H_2O (60:40, v/v) formulations with (▲) 1 DS, (●) 2 DS, and (■) 3 DS with 1% HPC, under finite dose conditions. Each data point represents the mean ± SD (n = 4–8).

HPC as an anti-nucleant agent did not improve the drug permeation from the formulation. As expected, the permeation from the subsaturated 0.5 DS formulation is also nearly half that of the saturated solution. These results suggest that the lack of permeation enhancement for the supersaturated formulation (even stabilised with polymer) is an indication that the residual phase is no longer supersaturated. This results from drug crystallisation as result of the solvent depletion (permeation or evaporation of PG). Drug crystallisation on or in the membrane has also been attributed to the lack of permeation enhancement for supersaturated formulations of oxybutinin in silicone and in skin (Santos et al., 2009, 2010). The findings are also in line with Nicoli et al. (2009) who investigated the dermatopharmacokinetics of ibuprofen when applied to human subjects using PG/H_2O vehicles. The authors demonstrated that PG losses reflected both diffusion through skin as well as evaporative loss to the atmosphere and that PG diffused more rapidly than ibuprofen thus "stranding" the drug in the stratum corneum. When evaporative loss of PG was prevented, by occlusion, ibuprofen remained able to diffuse from the stratum corneum.

Several enhancers have shown a concentration dependent permeation enhancement (Berner et al., 1989; Kurihara-Bergstrom et al., 1990; Smith and Maibach, 1995; Watkinson et al., 2009). A correlation between the concentration of PG in the formulation and the drug permeation through skin has also been shown for finite dose studies (Trottet et al., 2004). The results confirm that the maintenance of a diffusion source of drug is very important in transdermal drug delivery as solvent depletion promotes drug crystallisation and may decrease the membrane permeability. As a result, the drug partition and the diffusion coefficients may vary during the diffusion, and the permeation flux decreases with time. The results are also in line with those of Boix et al. (2005) who noted that depletion of terpenes from the skin induced changes in membrane permeability with time, which prevented the use of diffusion models to describe drug permeation.

4. Conclusions

In vitro studies using human skin were conducted to examine the mass transport of fentanyl from subsaturated, saturated and supersaturated PG/H_2O formulations. The addition of polymer (1%, w/v HPC) was shown to stabilise supersaturated formulations (3 DS) with increased drug flux through skin. The application of supersaturated formulations under finite dose conditions did not result in any further improvement in drug permeation with respect to control, even when a polymer was added to improve the formulation stability. However, infinite dose studies with the same supersaturated formulations prepared by mixing cosolvents did show an increase in drug permeation with increasing DS. The only difference between both studies (i.e., finite and infinite dose studies) is the donor reservoir capacity for drug and solvent. The lack of permeation enhancement with supersaturation observed for finite dose studies suggests that the PG had depleted from the donor compartment and consequently drug crystallisation on and in the membrane occurred. The importance of solvent depletion on drug crystallisation will be explored further in a future publication.

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