



Enhanced permeation of fentanyl from supersaturated solutions in a model membrane

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ARTICLE INFO

Article history:

Received 31 October 2010

Received in revised form 10 January 2011

Accepted 13 January 2011

Available online 21 January 2011

Keywords:

Supersaturation

Fentanyl

Permeation

Silicone

Propylene glycol

Ethanol

ABSTRACT

The aim of the present study was to investigate the permeation of fentanyl from supersaturated formulations when applied to silicone membrane. Silicone was chosen in order to separate the effects of supersaturation from other possible influences of volatile formulation components on biological membranes. Supersaturated formulations containing either propylene glycol/water (PG/H₂O) or propylene glycol/ethanol (PG/Et) were prepared containing varying degrees of saturation (DS) of fentanyl. Permeation of finite and infinite doses of the PG/H₂O formulations, and finite doses of the PG/Et formulations was investigated using Franz-type diffusion cells. For the PG/H₂O formulations a good correlation between the flux and the DS of the formulation up to 5 DS for infinite dose studies ($r^2 = 0.99$), and up to 7 DS for finite dose studies ($r^2 = 0.98$), was evident. Similarly, for the PG/Et formulations there is a good correlation between the mean flux and the theoretical DS of the formulation ($r^2 = 0.95$). Except for the 2 DS formulations, no significant differences were seen in the mean flux between PG/H₂O and PG/Et finite dose studies. The larger fluxes observed for infinite doses of the PG/H₂O formulations versus finite doses reflect changes in the effective area of diffusion over the time of the experiment for the latter set of experiments. The permeation enhancement observed for PG/Et formulations confirms that enhanced drug thermodynamic activity was induced by ethanol evaporation.

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1. Introduction

Currently marketed transdermal drug delivery systems rely on the use of physical methods and/or chemical permeation enhancers (CPEs) to improve drug permeation. Physical methods such as microneedles, electroporation or sonophoresis deliberately damage the skin barrier (Guy, 1996; Vanbever and Preat, 1999) while some CPEs (such as dimethyl sulphoxide or oleic acid) are known to give rise to localised irritation (Barry, 1987; Smith and Maibach, 1995). Supersaturation is an alternative to these permeation enhancement techniques, as it is a mechanism that does not change the barrier properties of the skin (Hadgraft, 2001). Nonetheless, the instability of supersaturated formulations is an important problem that is yet to be fully addressed. When a supersaturated solution is applied on the skin, the superficial layers of the SC will also be supersaturated (Hadgraft, 2004). As a result, increases in drug permeation will reflect the thermodynamic activity of the drug in the formulation.

Supersaturated systems may be obtained by solvent evaporation, mixing cosolvents, water uptake or rapid solubility changes

with temperature or pH (Davis and Hadgraft, 1993). The aim of the present study was to compare fentanyl permeation from supersaturated residues produced by (i) the cosolvent approach and (ii) solvent evaporation. Fentanyl was selected for study as it is currently delivered in patch form via the transdermal route. As the permeability coefficient of the unionised form is reported to be higher than the ionised form (Roy and Flynn, 1990), fentanyl base was used for all formulations. Formulations were prepared using either propylene glycol/water (PG/H₂O) or propylene glycol/ethanol (PG/Et). Using the two approaches, supersaturated formulations of varying degrees of fentanyl saturation (DS) were produced and evaluated for permeation through a silicone membrane. Silicone was selected as a model membrane in order to deconvolve the effects of supersaturation on permeant transport from the influence of formulation components on biological tissue.

2. Materials and methods

2.1. Materials

Fentanyl free base (racemic mixture) was a gift from Acrux, Ltd. (Melbourne, Australia). Propylene glycol (PG) and 1-heptane sulphonic acid were purchased from Sigma (Melbourne, Australia). Acetonitrile, methanol and water were HPLC grade. Ethanol 99%

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Table 1Fentanyl concentration and the respective DS of PG/H₂O and PG/Et formulations.

Formulation	DS	Fentanyl ($\mu\text{mol}/\mu\text{l}$)
60:40 v/v (PG: water)	1/2	0.011
	1	0.023
	2	0.045
	3	0.068
	5	0.113
	7	0.158
	1/2	0.138
60:40 v/v (PG: ethanol)	1	0.276
	2	0.553
	3	0.829

was purchased from Sigma (Melbourne, Australia). Deionised water was used to prepare PG/H₂O formulations. Silicone membranes with a thickness of 0.0125 cm were used for *in vitro* diffusion studies (Technical Products, Georgia, United States).

2.2. Solubility studies

Saturated solubility curves for fentanyl as a function of PG concentration in water were constructed. Saturated solutions were prepared by adding an excess of drug to solution. The samples were sonicated for 15 min and then equilibrated in a water bath at $32 \pm 1^\circ\text{C}$ for 24 h, under continuous stirring. At the end of this period, the solutions were centrifuged at 10,000 rpm/min at 32°C for 5 min (Eppendorf 5417R, Eppendorf International, Hauppauge, NY, United States). Using a calibrated micropipette, a small aliquot of supernatant (100 μl) was removed and diluted to 10 ml with methanol. This solution was further diluted with methanol, where necessary, before analysis.

2.3. Preparation and stability of supersaturated solutions

2.3.1. Propylene glycol/water (PG/H₂O) formulations

Supersaturation was produced using the cosolvent method described previously (Davis and Hadgraft, 1991). The composition of the formulations is given in Table 1. All solutions were filtered using a 0.22 μm filter (Millipore, Billerica, MA, United States). The ratio of the concentration of the drug in the formulation and the saturated solubility in the cosolvent mixture is the degree of supersaturation (DS).

For stability studies, supersaturated PG/H₂O formulations (60:40 v/v) with 2, 3, 5, and 7 DS were prepared. 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.

2.3.2. Volatile formulations

Subsaturated, saturated and supersaturated residues obtained by solvent evaporation were prepared (Table 1). The final formulation was filtered using a 0.22 μm filter.

2.4. Permeation studies

The permeation of fentanyl across silicone membranes was investigated using Franz-type diffusion cells, with a diffusional surface area of about 1 cm² and a receptor volume of approximately 3.4 ml (these values were accurately measured by weight for each cell and all results normalised accordingly). Small pieces of silicone with an area of $\sim 2\text{ cm}^2$ were prepared and soaked in ethanol overnight before use. A mixture of ethanol:water (20:80 v/v) was used as the receptor phase after degassing by sonication for 30 min. Infinite (250 $\mu\text{l}/\text{cm}^2$) and finite (25 $\mu\text{l}/\text{cm}^2$) volumes of donor solution were applied across the area of each piece of membrane using

a micropipette and spread with the pipette tip. Receptor phase samples were collected every 1–4 h over a 24 h period using an automated fraction collector (ISCO Retriever II, Lincoln, NE, USA). Prior to analysis, each vial was weighed on a Mettler AT261 analytical balance (Mettler Toledo GmbH, Schwerzenbach, Switzerland) and the volume calculated from the density of the receptor solution (0.997 g/ml at 22°C).

2.5. Solvent evaporation studies

Disks of silicone sheet with a diffusion area of 1 cm², the same as used for diffusion experiments, were placed on a plastic weighing pan. A Mettler AT261 precision weight balance connected to a personal computer using Labx® software (Applied Biosystems, Carlsbad, CA, United States) was used. The formulation (25 μl) was spread over the entire surface and the weight determined at different time intervals.

2.6. Area measurement

Digital pictures (Coolpix® 5200, Nikon Inc., Melville, NY, United States) were taken to measure the droplet area following application of 25 $\mu\text{l}/\text{cm}^2$ of formulation composed of 60% PG and 40% of ethanol or water on a piece of silicone membrane. The area of the droplet was calculated using Vistamatrix® software (Version 1.33.0, SkillCrest, LLC, Tucson, United States).

2.7. HPLC analysis

Fentanyl concentrations were determined using reverse phase high-performance liquid chromatography (HPLC) in conjunction with UV detection. The HPLC system consisted of a Waters Alliance HPLC System with a 2695 Separations Module and data were acquired and analysed using Empower® software (Waters, Milford, MA, United States). Fentanyl quantification was performed using a Waters Symmetryshield® C₁₈ column (5 μm particle size, $3.9 \times 150\text{ mm}$). A universal Waters Sentry® Guard with a C₁₈ cartridge was also used in conjunction with the column. The injection volume was 50 μl . A gradient method was used employing mobile phase A [10% v/v acetonitrile, 90% v/v water, 0.0001% v/v perchloric acid (70% v/v), 10 mM heptane sulphonic acid] and mobile phase B [100% v/v acetonitrile]. The mobile phase was pumped at a flow rate of 1 ml/min and the UV detection wavelength was 210 nm. The retention time for fentanyl under these conditions was $\sim 7.0\text{ min}$. Calibration curves for each assay were constructed using standard fentanyl solutions at concentrations within the range of 0.05–10 $\mu\text{g}/\text{ml}$. 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%.

2.8. Data analysis

2.8.1. Mean flux and ER

Linear regression was performed within the interval 20–35 min for diffusion studies performed with silicone in order to determine the mean flux (\bar{J}_{20-35}). The enhancement ratio (ER) is defined as the ratio between the mean flux of the supersaturated formulation under study and the mean flux of the saturated formulations.

Scientist® Version 3.0 (Micromath Inc., Salt Lake City, UT, United States) was used to fit diffusion equations to experimental data. Infinite and finite dose models expressed as Laplace transforms

were used (Eqs. (1) and (2)):

$$\overline{\text{Amount}} = \frac{\overline{\text{Flux}}(h)}{s} \quad (1)$$

$$\overline{\text{Amount}} = \frac{AP_1Q_0}{s[V\sqrt{(s/P_2)} \sinh \sqrt{(s/P_2)} + P_1A \cosh \sqrt{(s/P_2)}]} \quad (2)$$

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

$$P_1 = Kh \quad (3)$$

$$P_2 = \frac{D}{h^2} \quad (4)$$

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.

Using iterative procedures, permeation parameters were determined knowing the area (1 cm^2 for infinite dose studies, 0.32 and 0.37 cm^2 for finite dose studies using cosolvent and volatile formulations, respectively), the membrane thickness ($h = 0.0125 \text{ cm}$), the appropriate concentrations, the volumes applied and the ER. A minimum regression of 0.99 was obtained for all fitted data.

2.8.2. 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. All results are presented as the mean \pm SD, unless otherwise stated.

3. Results and discussion

3.1. Solubility studies

There is an exponential increase in drug solubility with increasing proportion of PG at 32°C (data not shown). This trend was previously described using the same cosolvent mixture but for other drugs such as hydrocortisone (Raghavan et al., 2001) and ibuprofen (Watkinson et al., 2009). The solubility of fentanyl in distilled water is $0.7 \pm 0.1 \mu\text{mol/ml}$, at 32°C , which is in line with values reported in the literature (Roy and Flynn, 1989; Roy et al., 1996). The solubility of fentanyl in pure PG is $461 \pm 33 \mu\text{mol/ml}$. Fentanyl is freely soluble in ethanol (Yum et al., 1994). Subsaturated, saturated and supersaturated solutions were subsequently prepared for PG/H₂O mixtures in the ratio 60:40 (v/v).

3.2. Stability

Fig. 1 shows the stability (or the remaining DS) of supersaturated formulations (2, 3, 5 and 7 DS) prepared by 60:40 v/v PG/H₂O vehicles over time. The remaining DS was calculated by dividing the drug still in solution by the solubility.

Formulations prepared with 5 and 7 DS were very unstable, as after 30 min the remaining DS was approximately 1.5. After 60 min, the drug had completely crystallised for both formulations (remaining DS = 1). The formulation with 2 DS shows good stability with an average remaining DS of 1.7 ± 0.2 over the 24 h of the study. Finally, the remaining DS for the formulation prepared with 3 DS, although lower than the theoretical value, was constant over 60 min, after which it started to crystallise thus reducing the DS.

3.3. Diffusion studies: silicone

In order to demonstrate that solvent evaporation from ethanol-based formulations enhances drug permeation by achieving

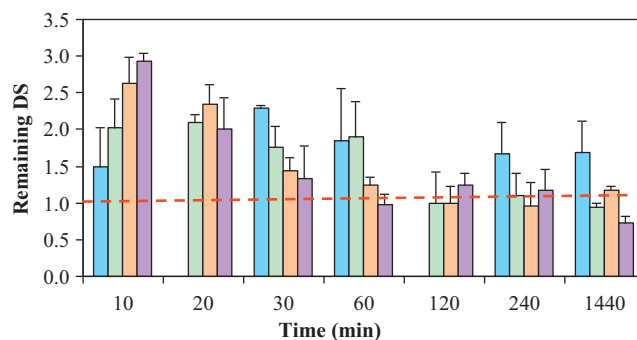


Fig. 1. Remaining DS of fentanyl in 60:40 PG/H₂O formulations with time. Legend: (■) 2, (■) 3, (■) 5 and (■) 7 DS of fentanyl. Red dashed line indicates 1 DS. Each data point represents the mean \pm SD ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

supersaturated states, the mean fluxes ($\bar{J}_{20-35'}$) achieved by supersaturated cosolvent and volatile solvent formulations were analysed and compared, *in vitro*, using silicone membranes

3.3.1. Cosolvent formulations

Fig. 2 illustrates the drug permeation through silicone membranes after the application of infinite ($250 \mu\text{l/cm}^2$) or finite ($25 \mu\text{l/cm}^2$) doses of saturated (1 DS) and supersaturated cosolvent formulations (2, 3, 5, and 7 DS). With the exception of the infinite dose studies with the 7 DS formulation, both permeation profiles show an increase in fentanyl permeation with increasing DS as a result of the increased thermodynamic activity of the drug.

Table 2 lists the calculated mean flux between 20 and 35 min $\bar{J}_{20-35'}$ and the ER for infinite and finite dose studies. No value of ER was obtained for the infinite dose study performed with 7 DS because of the marked curvilinear permeation profile for this formulation. This atypical profile is an indication of crystallisation as a result of formulation instability, and is in agreement with the stability data (Fig. 1). Raghavan et al. (2000) observed

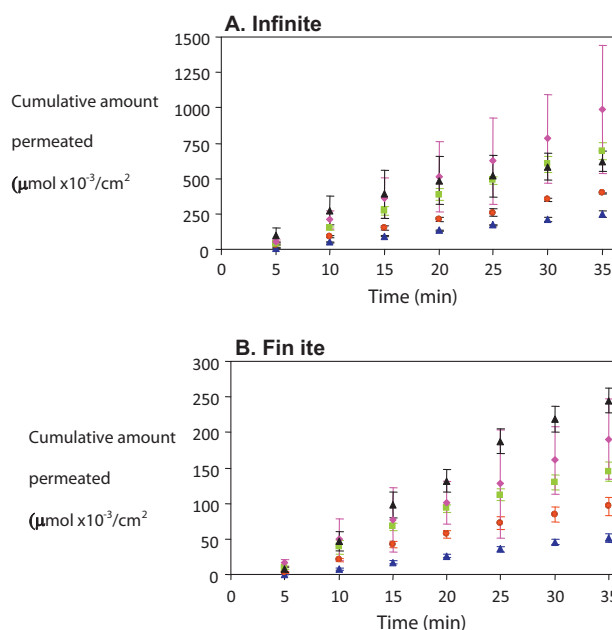


Fig. 2. (A) Permeation of fentanyl in silicone after application of $200 \mu\text{l/cm}^2$ of (■) 1 DS, (■) 2 DS, (■) 3 DS, (■) 5 DS and (■) 7 DS formulations composed of PG/water (60:40 v/v). (B) Permeation of fentanyl in silicone after application of $25 \mu\text{l/cm}^2$ of solutions of (■) 1 DS, (■) 2 DS, (■) 3 DS, (■) 5 DS and (■) 7 DS formulations composed of PG/water (60:40 v/v). Each data point represents the mean \pm SD ($n = 3$).

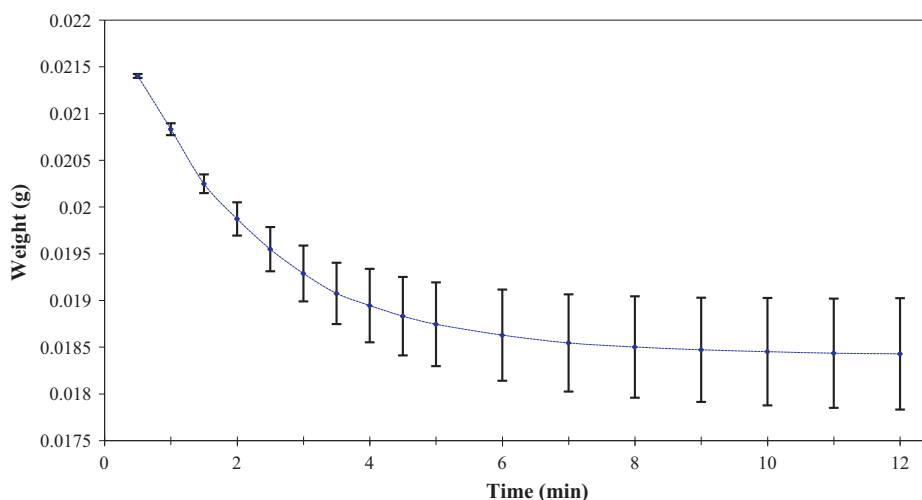


Fig. 3. Weight loss as result of ethanol evaporation with time following application of 25 $\mu\text{l}/\text{cm}^2$ of PG:Et (60:40 v/v). Each data point represents the mean \pm SD ($n=3$).

the same non-linear transport of hydrocortisone acetate in silicone membrane from 6.9-fold supersaturated gelled formulations of PG/H₂O (20:80 v/v). The decrease in the driving force as a result of drug crystallisation was also the main reason identified by Iervolino et al. (2000) to explain similar behaviour as well as high variability in the permeation observed with formulations with high DS. Finite and infinite dose studies showed similar levels of enhancement (Table 2), but the actual ER values were lower than the DS of the formulation. This is a further indication of instability problems associated with supersaturated states such as crystallisation or nucleation of the drug on and inside the membrane (Hou and Siegel, 2006).

A good correlation between the flux and the DS of the formulation up to 5 DS for infinite ($r^2=0.99$) and up to 7 DS for finite dose studies ($r^2=0.98$) is evident, despite the instability observed for formulations higher than 3 DS after 30 min. One of the reasons for this proportionality is the short duration of the experiments. As a result, at the end of the experiment, the unstable formulations are still supersaturated but with a lower DS than the predicted value, thus also explaining the lower ER value obtained compared with the DS of the formulation.

A correlation between the DS (for subsaturated and supersaturated formulations) and transport through artificial membranes is well documented with other drugs and cosolvent formulations (Davis and Hadgraft, 1991; Pellett et al., 1994; Schwarb et al., 1999; Iervolino et al., 2000; Raghavan et al., 2000). Davis and Hadgraft (1991) showed that the transport of hydrocortisone acetate across silicone from PG/H₂O vehicles with the same drug concentration increased with the DS of the formulation. In a later study, Pellett et al. (1994) showed that in PG:H₂O (60:40 v/v) formulations the

flux of piroxicam across silicone membranes was proportional to the DS of the formulation, up to 4 DS.

3.3.2. Formulations prepared by solvent evaporation

For the preparation of supersaturated solutions by solvent evaporation, formulations composed of ethanol (40% v/v) as the volatile solvent and PG (60% v/v) as the non-volatile solvent were used. Only finite dose studies were conducted, to ensure rapid evaporation of ethanol. Fig. 3 shows the weight loss as result of the ethanol evaporation when 25 $\mu\text{l}/\text{cm}^2$ of the formulation is applied to silicone membrane under non-occlusive conditions. The ethanol takes approximately 6–8 min to completely evaporate.

Fig. 4 shows fentanyl permeation after the application of 25 $\mu\text{l}/\text{cm}^2$ of PG:Et formulations. The drug permeation from supersaturated residues (2, 3, 5 and 7 DS) is higher than the saturated residue and increases with the drug concentration ($p<0.05$). These results suggest that the transport enhancement is the result of an increase in the thermodynamic activity, as the PG content is the same and PG does not interact with the membrane (Santos et al., 2009).

Table 3 lists the mean flux (\bar{J}_{20-35}), and ER for all PG:Et formulations. The ER and the mean flux increase with the theoretical DS of the formulation ($r^2=0.95$). The ER was lower than the DS of the formulation, which suggests a decrease in the drug thermodynamic activity with crystallisation. Except for 2 DS formulations, no significant differences were seen in the mean flux between PG/H₂O and PG:Et finite dose studies (Tables 2 and 3, respectively).

Table 2

Fentanyl mean permeation flux between 20 and 35 min (\bar{J}_{20-35}) and enhancement ratio (ER) from application of infinite or finite doses of PG:H₂O (60:40 v/v) formulations. Each value represents the calculated mean \pm SD ($n=3$). Note: uncorrected area was used for calculation of the flux, i.e. 1 cm^2 .

DS	PG/H ₂ O (60:40 v/v)			
	Infinite		Finite	
	\bar{J}_{20-35} ($\mu\text{mol}/\text{cm}^2/\text{h}$)	ER	\bar{J}_{20-35} ($\mu\text{mol}/\text{cm}^2/\text{h}$)	ER
1	0.46 \pm 0.07	1.0	0.10 \pm 0.02	1.0
2	0.79 \pm 0.09	1.7	0.16 \pm 0.02	1.6
3	1.23 \pm 0.22	2.7	0.22 \pm 0.04	2.2
5	1.91 \pm 0.79	4.1	0.36 \pm 0.04	3.7
7	–	–	0.46 \pm 0.02	4.6

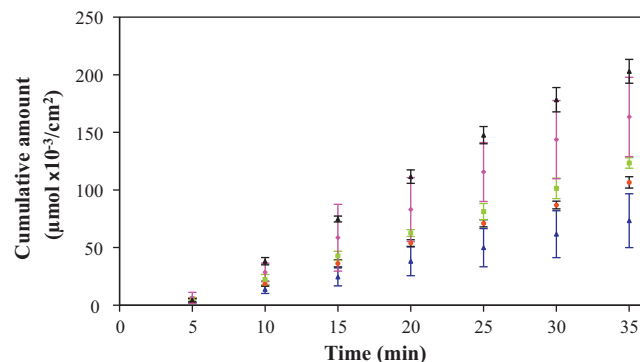


Fig. 4. Permeation of fentanyl across silicone after application of 25 $\mu\text{l}/\text{cm}^2$ of solutions with (▲) 1 DS, (●) 2 DS, (■) 3 DS, (◆) 5 DS and (▲) 7 DS, composed of PG:Et (60:40 v/v). Each data point represents the mean \pm SD ($n=3$).

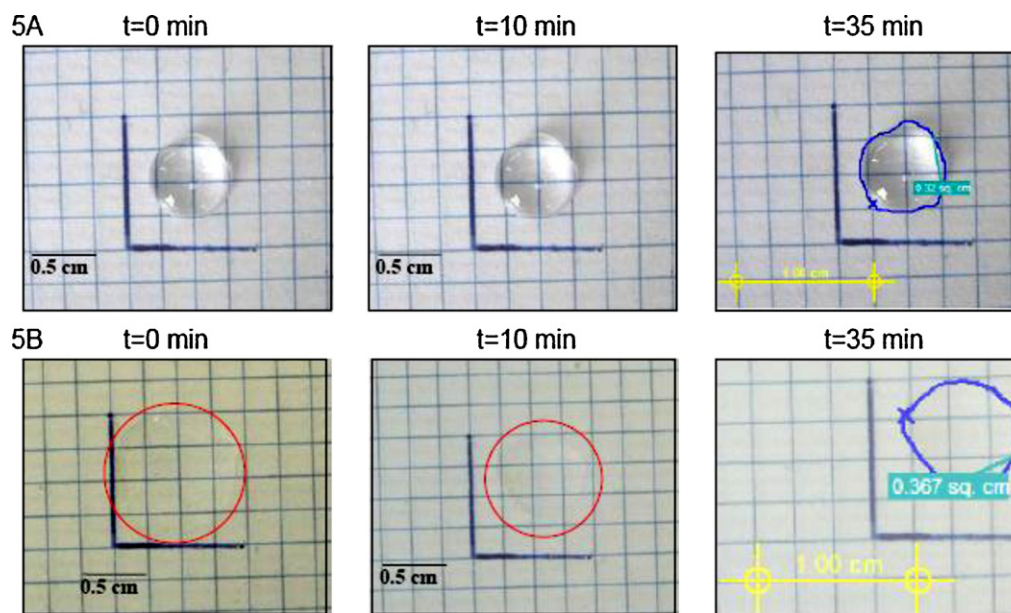


Fig. 5. (A, top) Droplet size area resulting from the application of $25 \mu\text{l}/\text{cm}^2$ of a formulation with 60:40 v/v PG:water, on a silicone membrane. (B, bottom) Changes in the droplet size area with the ethanol evaporation through time, following application of $25 \mu\text{l}/\text{cm}^2$ of 60:40 v/v PG:ethanol on a silicone membrane.

3.3.3. Flux correlation between finite (PG/H₂O and PG/Et) and infinite dose studies

Theoretically, using similar formulations the flux of a permeant should depend on the thermodynamic activity of the drug in the formulation as the membrane permeability remains constant. Therefore, in principle, saturated residues prepared with ethanol should produce the same flux as the PG/H₂O formulations with the same activity, as both water and PG are non interactive solvents for silicone membranes (Santos et al., 2009). In addition, silicone was soaked overnight in ethanol in order to minimise the interaction of ethanol from the PG/Et formulations with the membrane. The drug permeation should also be similar between finite and infinite dose studies for the same formulation and drug activity.

However, from Fig. 2, it is evident that this is not the case. Infinite dose studies produced considerably higher drug permeation than finite studies with the same DS. These unexpected results might be the result of differences in the diffusion areas depending on the volume of formulation applied. Therefore, the droplet areas, as well as area changes with evaporation, following application of $25 \mu\text{l}/\text{cm}^2$ of PG/H₂O and PG/Et formulations were investigated.

Fig. 5(A) shows the variation in the droplet size resulting from the application of $25 \mu\text{l}/\text{cm}^2$ of PG/H₂O formulations, at time 0, 10 and after 35 min. The high surface tension prevents the droplet spreading on the surface. Using Vistametric®, the area measured was approximately 0.32 cm^2 and remains fairly constant over time. Similarly, Fig. 5(B) shows the variation in the droplet size with ethanol evaporation after 10 and 35 min. With PG/Et formulations, although it was possible to spread the formulation all over the membrane surface, the residue formed after ethanol evaporation

did not cover the entire surface. Using Vistametric®, the calculated area of the residue was 0.37 cm^2 , slightly higher than that for the PG/H₂O area.

The results in Fig. 5 clearly show that the membrane surface (1 cm^2) is not completely covered by the formulation under finite dose conditions. As a result of changes in the effective area covered by the formulations, the permeation fluxes reported in Tables 2 and 3 were corrected accordingly. Fig. 6 shows the corrected mean flux of fentanyl permeation through silicone following the application of infinite or finite doses of PG/H₂O supersaturated formulations, and following the application of finite doses of PG/Et formulations.

From Fig. 6 it is evident that, after correction for the area of application, no considerable differences were seen in fluxes for infinite and finite dose studies for PG/H₂O formulations with the same DS.

For PG/Et formulations, with the exception of formulations prepared with 7 DS, no statistical differences ($p > 0.05$, ANOVA) were seen between the supersaturated residues prepared by the cosolvent approach or by solvent evaporation, despite the 12-fold difference in fentanyl concentration. The flux increases proportionally with the DS for all formulations. These findings demonstrate clearly that PG:Et formulations produce supersaturated residues with similar permeation fluxes as PG/formulations prepared with the same DS.

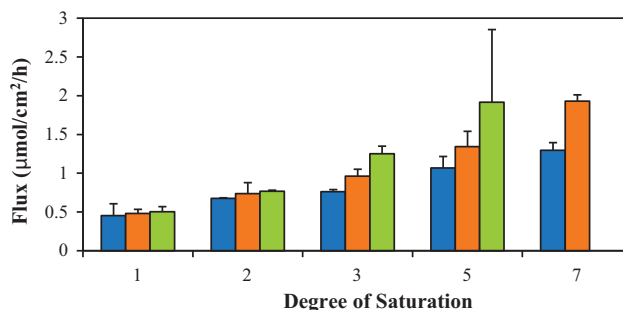


Fig. 6. Permeation fluxes of fentanyl through silicone membranes after application of saturated and supersaturated solutions, for finite doses of PG/Et (60:40) (■), and finite dose (■) and infinite dose (■) conditions for PG/H₂O (60:40). Each data point represents the mean \pm SD ($n = 3$). The fluxes were corrected for the effective diffusion areas calculated from Fig. 5.

Table 3

Fentanyl $\bar{J}_{20-30'}$ and enhancement ratio (ER) from the application of finite doses of PG/Et (60:40) formulations. Each value represents the calculated mean \pm SD ($n = 3$).

	PG/Et (60:40 v/v)	
	$\bar{J}_{20-30'}$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	ER
1 DS (control)	0.13 ± 0.04	1.0
2 DS	0.23 ± 0.01	1.8
3 DS	0.25 ± 0.02	2.0
5 DS	0.34 ± 0.02	2.7
7 DS	0.40 ± 0.03	3.2

Table 4, Supplementary data lists the diffusion parameters and calculated variability determined using appropriate finite and infinite diffusion models. One of the major problems in permeation parameter determination from the application of supersaturated formulations is the knowledge of the drug concentration (or the DS) because of the instability of these systems. In order to overcome this problem, the flux ER was used as an approximation of the concentration and these values were assumed to be constant over the experiment. High variability is evident for permeation parameters obtained for supersaturated formulations, especially for finite dose studies. This is a consequence of the effective area changes during diffusion. Less variability is observed in parameters for infinite dose studies. There are no differences in the permeation parameters (K and D) at different drug activity for these formulations, which indicates that the DS has no impact on the membrane permeability. The t_{lag} is approximately 3.5 min and the partition coefficient 0.55.

4. Conclusions

An *in vitro* study using silicone membranes was conducted to examine the mass transport of fentanyl from subsaturated, saturated and supersaturated residues obtained using the cosolvent approach or by using a volatile solvent. Drug permeation from residues with different DS produced by solvent evaporation is similar to the permeation from cosolvent formulations with the same DS. The permeation enhancement observed with ethanol-based formulations clearly results from an increase in the drug thermodynamic activity with ethanol evaporation.

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. These studies will be extended to human skin to investigate how the actual formulation components may impact on the barrier function and how these effects may modulate any permeation enhancement properties observed in the current work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.01.025

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