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Effect of finite doses of propylene glycol on enhancement of in vitro percutaneous permeation of loperamide hydrochloride

L. Trottet [a,*], C. Merly [a], M. Mirza [a], J. Hadgraft [b], A.F. Davis [a]

[a] *GlaxoSmithKline Consumer Healthcare, Weybridge, Surrey, UK*
[b] *Medway Sciences—University of Greenwich, Chatham Maritime, Kent, UK*

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Abstract

We hypothesised that the depletion of propylene glycol from topical formulations applied at clinically relevant doses (~mg/cm^2) would limit its penetration enhancement effect. The in vitro percutaneous permeation of a model drug—loperamide hydrochloride—in formulations containing propylene glycol was therefore investigated under finite dose conditions. The flux of loperamide and propylene glycol across dermatomed human skin was measured. The first study examined the effect of topical loading of a gel containing 12% propylene glycol. The second study investigated the effect of propylene glycol content in creams containing 15 and 40%. Both studies showed a correlation between the amount of propylene glycol dosed on the skin and the amount of drug that had permeated. The substantial permeation of propylene glycol and relatively small permeation of loperamide, strongly suggests, that the time dependent permeation of the drug was due to the depletion of propylene glycol at the skin surface and not to the depletion of the drug itself. As often doses applied in in vitro skin permeation experiments do not match the intended clinical dosage—they are usually much greater—this study suggests that the penetration enhancement effect of propylene glycol can be overestimated in in vitro studies.

Keywords: Propylene glycol; Loperamide; Percutaneous permeation; Finite dose; In vitro

1. Introduction

Propylene glycol (PG) is widely used as a penetration enhancer in topical dermatological preparations, either alone or in combination with other penetration enhancers, such as fatty acids (Bendras et al., 1995). Its proposed mechanism of action is to partition into the stratum corneum and increase permeant solubility in and thus permeant flux through the stratum corneum. From this, the flux of both propylene glycol and the permeant should be related and studies have showed such correlations (Polano and Ponec, 1976; Mollgaard and Hoelgaard, 1983; Wotton et al., 1985; Squillante et al., 1998).

However, demonstrating this was performed with relatively large applied doses of propylene glycol either due to the amount of formulation applied (greater than 30 mg/cm^2) or with formulations containing a high concentration of propylene glycol (greater than 50%). In contrast, most commercial formulations contain propylene glycol in a range of 5–50%, usually 5–20%. Also, in clinical use, topical vehicles are applied at lower doses than 30 mg/cm^2, and this will vary depending on the application. Data on the dose applied in clinical situations suggest that the amount

* Corresponding author. Present address: GlaxoSmithKline, Les Ulis 91940, France. Tel.: +33-1-69-29-6031; fax: +33-1-69-29-6060.
E-mail address: lionel.x.trottet@gsk.com (L. Trottet).

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of formulation applied depends on the body surface area treated: the larger the surface area, the lower the amount of product applied is. For a cold sore, a very small surface area is generally treated (~2 cm^2), and a 2 g tube will last on average for two cold sore episodes (five applications per day for 5 days). This leads to an average amount applied of 20 mg of product/cm^2. For skin diseases involving larger body surface area, Surber and Davis (2002) review the field related to the dose applied with topical formulations. They show that the amount applied vary from 0.7 to 4 mg/cm^2. Interestingly for sunscreens where most of the body is treated, the amount applied falls down to 0.5 mg/cm^2 (Bech and Wulf, 1992; Azurdia et al., 1999).

Based on the physicochemistry of propylene glycol and predictions of its flux we hypothesised that the flux of propylene glycol would be sufficient to cause depletion of propylene glycol from typical commercial vehicles applied at clinically relevant doses. Because of the relationship between the flux of both propylene glycol and the permeant we further hypothesised that permeant flux would be dependent upon propylene glycol dose under conditions of finite dose of propylene glycol. Smith and Maibach (1995), in a review of penetration enhancers, have been concerned that apparent effects of penetration enhancers could be dependent upon their dose. However, little is found in the literature on effect of dose of penetration enhancers on permeant penetration despite different guidelines advising that in vitro skin permeation studies should be performed using the clinical intended dosage (Skelly et al., 1987; Howes et al., 1996; Diembeck et al., 1999).

In this study, the permeation of propylene glycol and of loperamide hydrochloride, a hydrophobic basic model compound, was studied from gel and cream formulations with the aim to identify the effect of propylene glycol dose on the loperamide permeation under clinically relevant conditions.

2. Materials and method

2.1. Materials

[^{14}C]Loperamide HCl (NEN, Boston, MA), used as received, had a radiochemical purity of 99.6% and a specific activity of 54 mCi/mmol. [^{14}C]Propylene glycol (ICN, Irvine, CA) had a radiochemical purity of 97% (because of the evaporation potential of propylene glycol, the total dose of [^{14}C]propylene glycol used was limited to 7 μCi). Non labelled loperamide HCl was purchased from Sigma. The components of gel and creams are described in Tables 1 and 2, respectively (the co-solvent mixture is proprietary but was kept constant and is therefore not a variable that needs to be taken into consideration in these experiments).

2.2. Methods

2.2.1. Formulations tested

The gel and cream formulations are described in Tables 1 and 2. They were made firstly with the drug

Table 1
Gel composition

Formulation components	% w/w
Loperamide HCl	3
Propylene glycol	12
Co-solvent 1[a]	10
Co-solvent 2[a]	19
Co-solvent 3[a]	48
Silica (thickener)	8
Total	100

[a] Co-solvents 1, 2 and 3 mixture are proprietary. Co-solvents 1, 2 and 3 are non-volatile.

Table 2
Cream compositions

Formulation components	% w/w	
	Cream 15% PG	Cream 40% PG
Loperamide HCl	2.5	5
Propylene glycol	15	40
Co-solvent 1[a]	5	5
Co-solvent 2[a]	2	2
Co-solvent 3[a]	13	13
Benzyl alcohol	1	1
Stearyl alcohol	5	5
Surfactants	2.2	2.2
Thickener	0.8	0.8
Buffer system	4	4
Water	49.5	22
Total	100	100

[a] Co-solvents 1, 2 and 3 mixture are proprietary. Co-solvents 1, 2 and 3 are non-volatile.

as the radioactive marker and then with propylene glycol as the radioactive marker. Only radiolabelled drug was used (i.e. no spiking) during the [^{14}C]loperamide cream and gel manufacture.

To understand further the steady state permeation of propylene glycol, a solution of 50/50 [^{14}C]propylene glycol/water (pH 4) [potassium hydrogen phthalate (Sigma) at 0.05 M in water] was prepared.

It is noteworthy that saturated solubility studies were conducted for all the formulations and their different phases, to ensure that sufficient loperamide was added to the formulations to ensure that the drug was in suspension before the formulation was applied onto the skin. Saturated solubility studies were conducted by adding loperamide to the solvent mixture of interest in an eppendorf. After overnight mixing, the sample was centrifuged for 5 min at 10,000 rpm and the supernatant was analysed by High Pressure Liquid Chromatography (HPLC).

2.2.2. Diffusion studies

All formulations were tested following the same protocol. Bronaugh type flow through diffusion cells (Permegear—USA), having an available diffusion area of 0.64 cm^2 and a receptor volume of 0.3 ml, were employed (Squier et al., 1997). In the two studies, the same dermatomed (∼400 μm) human (back) cadaver skin (stored at −20 °C after collection till used) from one single donor (55-year-old—female) was used. Cut skin samples were placed in the diffusion cells. The flow through system was then left to equilibrate for approximately 2 h prior to application. The receptor phase (phosphate buffer saline, pH 7.4, Sigma) was pumped at a rate of 1.5 ml/h, five or six replicates were used per formulation. Accurately weighed quantities of gel or creams were applied to the surface of the skin and spread by means of a small bent metal spatula. Because of practical reasons (small surface area of the diffusion cell), it was considered unreasonable to load less than 10 mg/cm^2. Therefore the dose loading applications were of 10 or 40 mg/cm^2. The permeation at 40 mg/cm^2 compared with a 10 mg/cm^2 loading was used to estimate what would occur at the more clinically relevant permeation if the 10 mg/cm^2 loading was decreased to 2 mg/cm^2—assuming linearity across this whole range of dose loading. In these finite dose applications, the cells were left unoccluded. For the 50/50 [^{14}C]propylene glycol/water (pH 4) control solution, 400 μl of solution was applied with a pipette and the cell was then occluded. Samples were automatically collected in scintillation vials at 2- or 4-h intervals for 24 h.

Eight millilitres of scintillation cocktail (Hi Phase Supermix—EG&G Wallac, UK) was added to the samples and samples were analysed with a Rack Beta 1209 scintillation counter (EG&G Wallac). For the study with [^{14}C]propylene glycol, recovery of propylene glycol in donor and in skin was carried out at the end of the study. At 24 h, the donor compartments of the diffusion cells were washed with 400 μl of Transcutol (Gattefossé)—used for its intermediate polarity between propylene glycol and the three co-solvents used—followed by two washes of 400 μl of water and then wiped with tissue. The diffusion cells were then dismounted and wiped again. The three washes and the two wiping tissue samples were pooled and 8 ml of scintillant was added and the sample analysed. The skin was then placed in a scintillation vial filled with 8 ml of water to extract the propylene glycol present in the skin (propylene glycol being a small molecule with high diffusing property as proved by its flux profile, as well as miscible with water, skin homogenisation was not required for its extraction). After overnight extraction, 8 ml of scintillant was added and the sample analysed.

2.2.3. Skin permeation data analysis

Because of the log normal distribution of skin permeability as described by Williams et al. (1992), flux data were analysed by the method proposed by the same authors. Error bars plotted represent standard deviation.

3. Results

With the gel formulation, the effect of dose loading on the flux of both the drug and propylene glycol was investigated. The flux profiles are presented in Fig. 1. For both permeants, the fluxes were roughly proportional to the dose applied (about four-fold difference). For both permeants, the permeation did not reach a steady state with a peak flux occurring earlier for the propylene glycol than for the loperamide. The shape of the flux curves seems to indicate that there is depletion of propylene glycol and possibly of the drug with

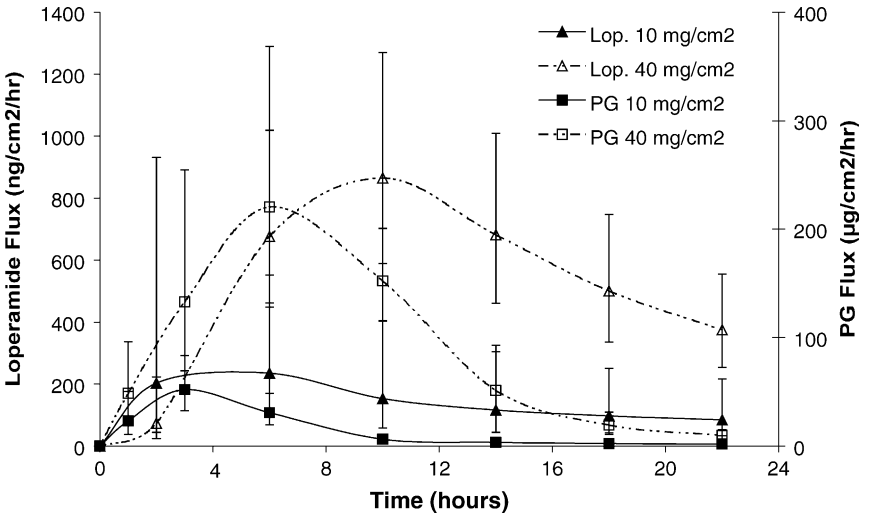

Fig. 1. Flux of loperamide and propylene glycol from the gel formulation. Closed triangle (▲): loperamide at 10 mg/cm^2. Closed square (■): PG at 10 mg/cm^2. Opened triangle (△): loperamide at 40 mg/cm^2. Opened square (□): PG at 40 mg/cm^2.

time. The mass balance of propylene glycol (Table 3) confirms the depletion of propylene glycol while less than 5% of the drug applied has permeated after 24 h. The permeation of propylene glycol is about 400-fold higher than the permeation of the drug.

The effect of propylene glycol content in the creams on the flux of both permeants is presented in Fig. 2. The fluxes of both permeants appeared to be proportional to the amount of propylene glycol present in the two formulations. As for the gel, dynamic (i.e. a steady state is not achieved) permeation is observed as well as an earlier peak for propylene glycol compared to the drug, and an apparent depletion of propylene glycol with time. Depletion of propylene glycol is confirmed by mass balance (Table 3) while less than 1% of the drug applied has permeated after 24 h.

If the same amount of cream or gel is applied on the skin, the amount of propylene glycol permeating through the skin is proportional to the amount of propylene glycol present in the formulation as demonstrated by the line drawn on Fig. 3: the three points corresponding to a 10 mg/cm^2 topical application fit a line going through the origin. The proportion of propylene glycol permeated is about 30% if 10 mg/cm^2 of topical is applied but increases to 45% if the amount applied increases to 40 mg/cm^2.

In the controlled environment (infinite + occluded condition) of the 50/50 propylene glycol/water solution (=at half its maximum saturation level), the flux of propylene glycol reaches steady state (Fig. 4). The mass balance of propylene glycol permeation is presented in Table 3. In the finite condition, the recovery of propylene glycol is not total and high variation in the donor compartment recovery is observed. On the other hand, in the infinite + occluded condition, the

Table 3
Propylene glycol mass balance

	PG/water 50/50 infinite dose (S.D.) ($n = 5$)	12% PG gel 10 mg/cm^2 (S.D.) ($n = 6$)	12% PG gel 40 mg/cm^2 (S.D.) ($n = 6$)	15% PG cream 10 mg/cm^2 (S.D.) ($n = 6$)	40% PG cream 10 mg/cm^2 (S.D.) ($n = 5$)
% dose in receptor	0.65 (0.35)	29.9 (8.5)	45.4 (5.4)	31.4 (5.2)	36.0 (15.4)
% dose in donor	95.9 (8.1)	27.4 (25.6)	30.6 (26.1)	4.2 (0.8)	14.3 (11.0)
% dose in skin	0.16 (0.03)	4.2 (1.1)	2.9 (0.4)	3.1 (0.6)	2.5 (1.1)
% dose in receptor + donor + skin	96.7 (8.5)	61.4 (35.1)	78.9 (31.9)	38.7 (6.6)	52.8 (27.6)
% missing	3.3	38.6	21.1	61.3	47.2

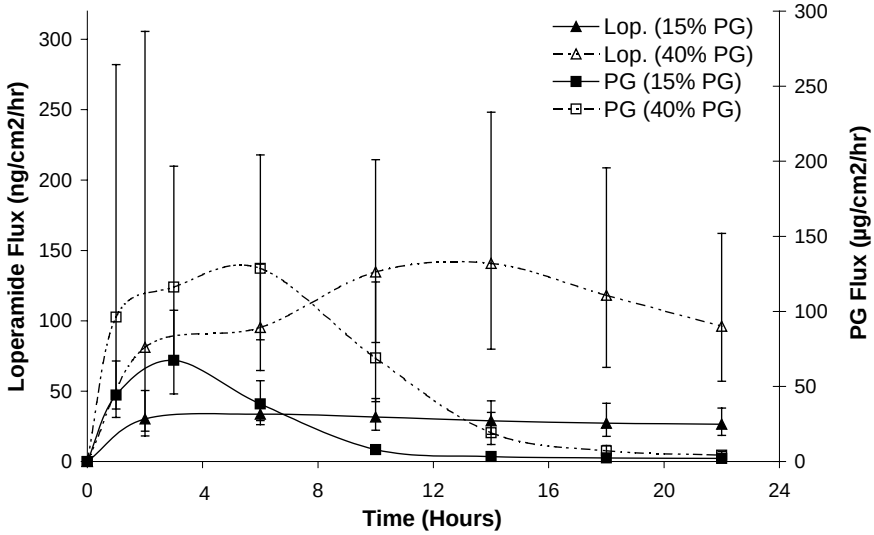

Fig. 2. Flux of Loperamide and propylene glycol from the cream formulations. Closed triangle (▲): loperamide in 15% PG cream. Closed square (■): PG in 15% PG cream. Opened triangle (△): loperamide in 40% PG cream. Opened square (□): PG in 40% PG cream.

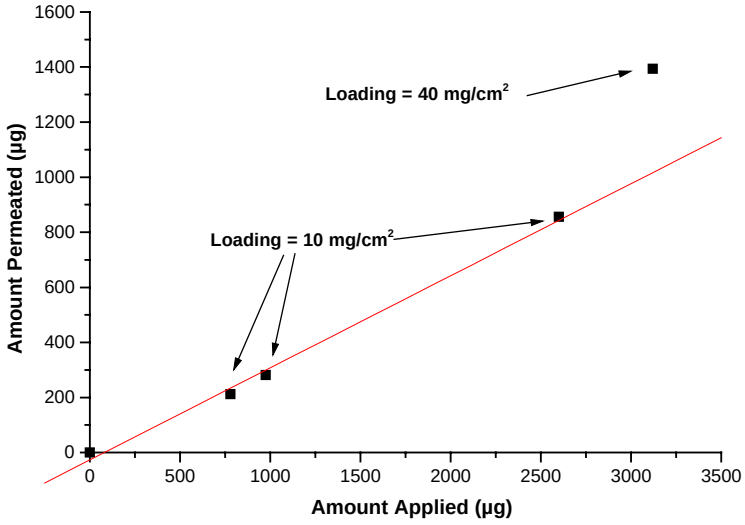

Fig. 3. Influence of the amount of topical applied on the percentage of propylene glycol permeated.

recovery was virtually 100% and only small variations were observed.

4. Discussion

The penetration enhancement effect of propylene glycol on the drug has been demonstrated and profiles of both permeants suggest strongly that it is the permeation of propylene glycol that drives the permeation of the drug. There are several indications for that: earlier peak flux of propylene glycol compared with the drug, depletion of propylene glycol in donor but not of the drug, penetration enhancement of the drug linked with propylene glycol dose loading.

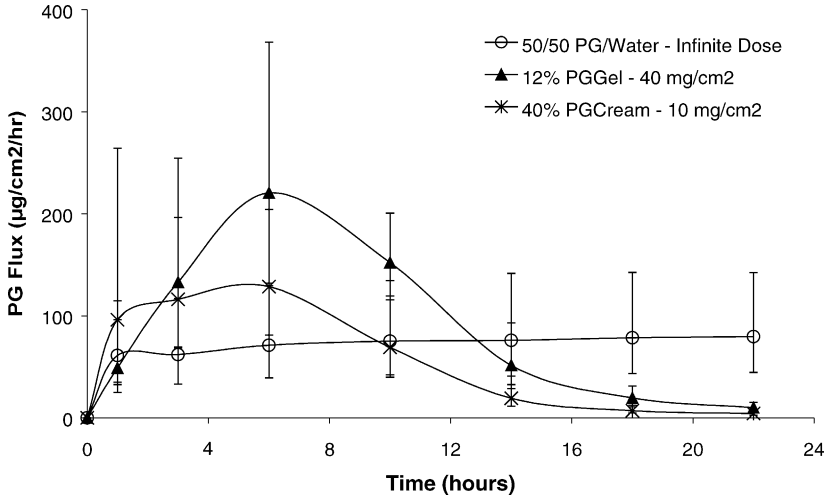

Fig. 4. Flux of propylene glycol from a gel, a cream and an aqueous solution. Open circle (○): 50/50 PG/water—infinite dose. Closed triangle (▲): 12% PG gel at 40 mg/cm^2. Star (✳): 40% PG cream at 10 mg/cm^2.

Beyond this permeation enhancement phenomenon, the comparison of flux magnitudes and flux profiles observed between propylene glycol and loperamide are of interest. First, there is a two to three order of magnitude difference in flux in between these two permeants. That is no real surprise as there is a large difference in the size of propylene glycol and loperamide, molecular weight 76 and 477, respectively, and lipophilicity and solubility characteristics. Empirical equations (Potts and Guy, 1992; Roberts et al., 1995; Pugh et al., 1996; Roberts et al., 1996) based on physiochemical determinants predicting the permeability coefficient and therefore the flux of molecules through skin often take into account the molecular weight, which is inversely related to permeability coefficient.

Secondly, the permeation profiles of the two molecules are different, propylene glycol crosses the skin barrier more quickly than loperamide, shown by the earlier peak of propylene glycol. It is interesting to make the analogy between a skin permeation experiment and HPLC as a similar phenomenon occurs. If the stratum corneum for skin permeation is compared to a reversed phase silica column for HPLC, the time required for a molecule to cross the stratum corneum matrix can be associated with the retention time observed for a molecule diffusing through the column silica matrix. In HPLC, with Ultra Violet analytical detection, the peak of the solvent in which the analyte was dissolved comes before the peak of the analyte, and this is similar to the observation in this skin permeation experiment. A large molecule is more likely to be "delayed" compared to a smaller one because of specific binding or interactions with the different elements of the stratum corneum.

The study of the results of the mass balance of propylene glycol fits well with the findings of Tsai et al. (1992) who studied the evaporation of propylene glycol from propylene glycol/ethanol/water mixtures. They showed that with a semi-finite loading dose, propylene glycol did evaporate. As well, they observed that the smaller the loading dose, the higher was the degree of evaporation. The results in our study suggest the same outcomes as not all the propylene glycol is recovered from donor + skin + receptor. Additionally, with the high loading dose of 40 mg/cm^2 only 25% of propylene glycol evaporated while for the lower dose of 10 mg/cm^2, more propylene glycol has: 39, 47 and 61%. This higher evaporation correlates with the amount permeated as shown in Fig. 3.

The high variability in the recovery of the donor is likely to be due to a practical issue as some gel/cream will not be applied to the surface of the skin but on the internal side of the donor chamber (difficulty in applying small dose on small surface area). This issue is likely to be minimised in permeation studies using

cells of larger surface area: diffusion cell design can be key to get better in vitro skin permeation studies at finite dose.

The effect of propylene glycol loading dose in these in vitro studies confirms the overestimation error suggested by Smith and Maibach (1995) if the in vitro study is not conducted with a clinically relevant dose. This emphasises the significance of formulation excipients in the optimisation of topical formulations. Perhaps more importantly, it is of significance in the experimental design of in vitro permeation studies. Those studies in which the dose applied is significantly superior to the clinical one can give erroneous and therefore misleading results.

It would be interesting to conduct further studies on other topical formulations with other penetration enhancers of the solvent type like propylene glycol to confirm these findings.

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