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Film drying and complexation effects in the simultaneous skin permeation of ketoprofen and propylene glycol from simple gel formulations

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Abstract

This work investigated the simultaneous permeation of ketoprofen and propylene glycol (PG) across pig ear skin from simple gel formulations administered under simulated in-use conditions. The aims were to quantify rates of permeation of both solvent and active, probe the effects of formulation drying and gain insight into drag/complexation interactions. Simple 3-component gels were formulated using a fixed amount of ketoprofen and hydroxypropyl cellulose thickener with decreasing content of solvent propylene glycol. Multiple finite (5 mg × 15 mg) doses were massaged over 24 h into full thickness pig ear skin in vertical Franz-type diffusion cells. The permeation of ketoprofen was inversely proportional to the content of PG, whereas the permeation of PG was directly proportional, although the amount of PG permeated was always greater than ketoprofen, even from the driest gel practically achievable. In this state, the molar ratio of PG/ketoprofen was ∼12, suggesting that this number of PG molecules constitutes the solvation cage of ketoprofen. Dragging/pulling effect extends throughout the skin and into the receptor compartment and probably the system, in an in vivo situation. Although PG may represent a worse case scenario given its well-documented skin permeation enhancement properties, it is probable that other solvents exert a similar effect on solutes across skin. A drying film will behave in different ways depending on the nature of both the thickener and solvent, where the outcomes are not readily predictable. It is important to account for the fate of all species administered from a topical formulation.

Keywords: Ketoprofen; Propylene glycol; Topical; Permeation; Drag effect; Solvated complex; Drying; Skin

1. Introduction

The application of dermatological formulations is a familiar experience for most people in Western societies, whereby a portion of material is massaged over an area of skin to form of an ultrathin film. However, contained within this apparently simple procedure are a number of issues that have not been fully addressed within the literature. Firstly, the processes occurring within a film post application (collectively referable to as 'drying') can have important ramifications for the efficacy of the applied medication. Part of the problem could be the difficulty in studying a dynamically changing film, although a 'snapshot' approach, in which specific time-points in the drying process are considered individually and which collectively mimic a changing formulation, was recently reported (Gallagher et al., 2003a; Gallagher et al., 2003b).

A further issue concerns a general misconception that for a given topical product only the active permeates the skin and that it will do so as discrete molecules, fully dissociated from all excipients. Skin permeation theory indicates that the higher the thermodynamic activity, the greater the potential of a drug to exit a formulation to commence the permeation process. However, if the applied film dries post application it must ipso facto be losing its solvent content and it is apparent that the solvent can only go in one or both of two directions: into the atmosphere or into the skin. The issue of absorption of solvent into the skin was recognized at least as far back as 1964, where dimethyl sulphoxide was found to penetrate biological membranes including skin (Horita and Weber, 1964). It has also been known for some time that propylene glycol (PG) is a potent skin penetration enhancer and studies involving the measurement of solvent and solute led to the proposition of the so-called 'drag' effect (Hoelgaard

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and Møllgaard, 1985; Bendas et al., 1995) where the solvent was believed to modulate the barrier of the skin allowing easier passage for the migration of the solute. However, recognition that such an effect can persist throughout the skin to the receptor phase is a more recent development (Squillante et al., 1998; Trottet et al., 2004) and this phenomenon has been specifically utilised as a novel mode of delivery in the simultaneous skin permeation of pharmacologically active oils and solute co-permeant drugs (Heard et al., 2003a,b; Karia et al., 2004). Moreover, it is a generally accepted phenomenon in solution chemistry that the dissolution of a compound (e.g. drug) within a solvent (e.g. vehicle) can give rise to persistent solvated complexes and evidence for the permeation of such complexes across skin has been postulated (Heard et al., 2003a,b; Karia et al., 2004) along with a potential mechanism based upon π–π complexation (Heard et al., 2005).

The current studies are concerned with simple 3-component gels comprised of ketoprofen, hydroxypropyl cellulose and PG. The proportion of PG varied markedly from 91 to 38% (the lowest amount that could be added to achieve a homogeneous gel) to model a drying film and the effects on the permeation of both ketoprofen and PG were studied through skin in vitro.

2. Materials and methods

2.1. Materials

Ketoprofen, propane 1,2 diol (propylene glycol or PG), hydroxypropylcellulose (HPC) and PBS sachets pH 7.4 were purchased from Sigma, Poole, UK. HPLC grade acetonitrile was from Fisher Scientific, Loughborough, UK. Ultrafree-CL 2-ml tubes, containing low-binding Durapore PVDF 0.22 μm filter inserts, were from Millipore, Bedford, MA, USA. Porcine ears were obtained from a local abattoir prior to steam cleaning and stored at −20 °C for ∼1 week prior to use.

2.2. Gel preparation

This work involved simple gels comprised of ketoprofen, hydroxypropyl cellulose thickener and propylene glycol, where the latter was varied to obtain a series of gels ranging from 'normal' consistency (mimicking the point of application or t_0) to the most viscous (dry) (Table 1). As such, the t_0 gel had the lowest concentration/thermodynamic activity in respect of ketoprofen, whereas the driest gel (V) had the highest. Pre-determined amounts of ketoprofen and PG were combined and placed in an ultra-sonic bath until complete dissolution achieved. Thickener was then added and worked until completely dispersed using a ceramic pestle and mortar. The gels were then sealed and left to stand for 24 h resulting in transparent, homogenous products.

2.3. Preparation of pig ear skin membranes

Pig ear skin is widely regarded as a suitable substitute for modelling human skin permeability (Simon and Maibach, 2000). The porcine ears were thoroughly defrosted and washed under running cold water, before being shaved using electric clippers. Using a scalpel, full thickness skin was carefully removed from the underlying cartilage, before being cut into 2 cm × 2 cm sections. The skin samples were used immediately.

2.4. In vitro transcutaneous permeation

Permeation experiments were performed using Franz-type cell receiver chambers, of 3 ml nominal receptor phase volume and diffusional area, 0.78 cm^2; fitted with 1 cm diameter × 1 mm thick zinc washers as donor compartments. Skin samples from the three donor pig ears, distributed equally between the treatments to avoid bias, were placed onto the pre-greased flanges of the receptor compartments and the donor chambers affixed using pinch clamps. Micro stirrer bars were added to the receptor compartments which were then filled with degassed PBS pH 7.4 and the cells placed on a multiple stirrer plate in a thermostatically controlled water bath set at 37 °C. The cells were then dosed (t_0) with 15 mg of gel by gently massaging into the skin surface using the flattened end of a glass rod with four circular motions. The rod was weighed before and after to confirm dosage level. The receptor phase sampling arms were capped, but the donor phases remained unoccluded. At 3, 6, 12, 24 and 48 h the entire receptor phase was removed using extended syringes and replaced with temperature-equilibrated PBS—this sampling method was employed to minimise the risk of receptor saturation. A further 15 mg of the relevant formulation was massaged into the skin using a glass rod at each sampling point (except 48 h), giving a total dose of (5 × 15) 75 mg of formulation over 24 h. The samples were subjected to ultracentrifugation, the ultrafiltrates transferred to autosampler vials and stored at −20 °C prior to analysis. Six replicates were carried out for each treatment.

2.5. Reverse phase HPLC analysis

HPLC analysis was performed using an Agilent 1100 HPLC automated system fitted with a Kingsorb 5 μm 250 mm × 4.6 mm C18 column (Phenomenex, Macclesfield, UK). Each sample was analysed twice. For ketoprofen the mobile phase consisted of 45:55 potassium phosphate solution (pH 3):acetonitrile, with UV detection at 258 nm. For PG the mobile phase was deioinised water and detection was at 190 nm, with the PG peak identified by analysing spiked samples (Gao et al., 2003). For both analytes a 20-μl injection volume and a flow rate of 1 ml min^{-1} was used. Retention times were 6.6 min for ketoprofen and 4.3 min for PG. Standard calibration curves were linear in both cases ($R^2 \geq 0.9998$) using standard solutions prepared in mobile phase. Limits of detection were 800 ng ml^{-1} for ketoprofen and 750 ng ml^{-1} for PG.

2.6. Data processing

HPLC data was corrected for sampling/dilution effects and cumulative permeation data determined in units of both mass and moles per unit area. Student's *t*-tests were performed using Microsoft Excel.

Table 1
Formulae of gels

	Gel I	Gel II	Gel III	Gel IV	Gel V
Ketoprofen	0.8140 g 2.16%	0.8140 g 2.74%	0.8140 g 3.65%	0.8140 g 5.85%	0.8140 g 14.75%
Hydroxypropyl cellulose	2.6048 g 6.93%	2.6048 g 8.78%	2.6048 g 11.67%	2.6048 g 18.71%	2.6048 g 47.20%
Propylene glycol	32.56 ml 34.188 g 90.91%	25.00 ml 26.25 g 88.48%	18.00 ml 18.9 g 84.68%	10.00 ml 10.5 g 75.44%	2.00 ml 2.1 g 38.05%

3. Results and discussion

3.1. Permeation of ketoprofen from gels with varying PG content

The purpose of dosing multiple 15 mg potions of formulation was to be representative in an in-use regimen, although each system will differ in practice. Fig. 1 shows that the permeation of ketoprofen increased in accordance with its increasing content within the gel (as a consequence of decreased solvent content). This is reflected in the cumulative permeation data, where at 24 h there was (1.35×10^{-2} mmol cm^{-2}/2.49×10^{-3} mmol cm^{-2}) ~5.4 times and at 48 h there was (3.19×10^{-2} mmol cm^{-2}/5.90×10^{-3} mmol cm^{-2}) ~5.4 times more ketoprofen permeated from the driest gel relative to the t_0 gel (Table 2). The increased permeation from going from t_0 to the driest gel (V) can be attributed to the increased concentration or potential of ketoprofen (apparent increase with the drying film scenario). This contrasts with earlier work involving Cabosil/PEG400/ketoprofen gels where diminishing solvent content resulted in reduced permeation of ketoprofen, as a consequence of substantial binding to the silica thickening agent (Gallagher et al., 2003a).

Fig. 1 also shows that permeation of ketoprofen from the t_0 and driest gels were both zero-order, with steady state being attained, unlike the three intermediate gels where depletion was evident. All cells were repeat-dosed in the same manner with a total of 75 mg formulation added. Given negligible binding to the thickener, the behaviour of ketoprofen in the driest gel is readily explained as it contained the highest concentration of ketoprofen among the test gels. However, the steady state attained with the t_0 gel (lowest ketoprofen concentration, lowest thermodynamic activity) appears aberrantly high, if considered in isolation from the permeation of the solvent.

3.2. Permeation of PG from gels with varying PG content

Fig. 2 shows that there was a trend whereby the greater the amount of PG the greater the permeation of PG. However, the trend was modest in that at 24 h there was (0.361 mmol cm^{-2}/0.195 mmol cm^{-2}) ~1.85 times and at 48 h there was (0.407 mmol cm^{-2}/0.318 mmol cm^{-2}) ~1.28 times more PG permeated from the t_0 gel relative to the driest gel (Table 3). However, the only statistically significant difference ($p \leq 0.05$) was between Gels I and V. The similarity in permeation of PG from Gels I to IV may indicate that they are essentially behaving similarly and that the skin had saturated with PG. Only when the amount of PG reduced markedly (Gel V) was a substantial drop in permeation of PG observed, possibly due to solubility in the skin ceasing to become rate-limiting. However, it is notable that even from the most viscous (driest) gel practically achievable, there was still substantial permeation of PG.

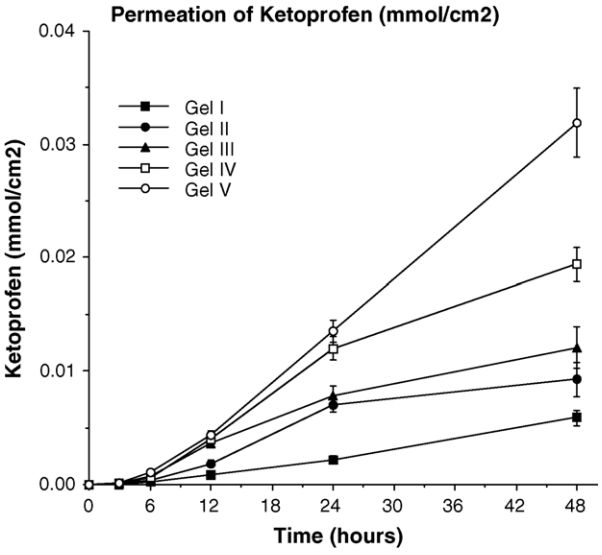

Fig. 1. Cumulative permeation of ketoprofen (mmol cm^{-2}) from five gels with varying propylene glycol content (I = 90.91%, II = 88.48%, III = 84.68%, IV = 75.44%, V = 38.05%), $n = 6$, ±S.E.M.

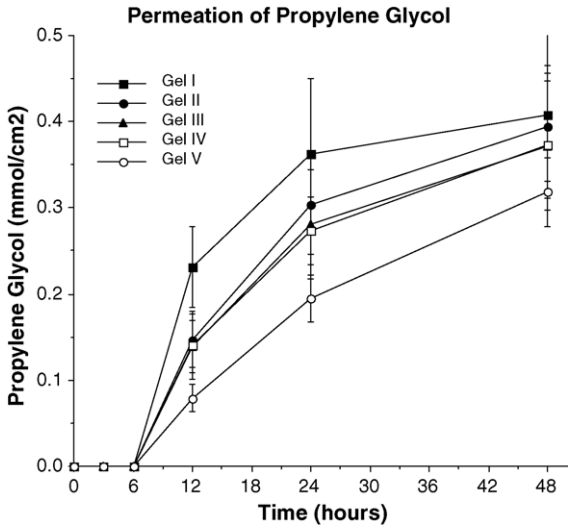

Fig. 2. Cumulative permeation of propylene glycol (mmol cm^{-2}) from five gels with varying propylene glycol content (I = 90.91%, II = 88.48%, III = 84.68%, IV = 75.44%, V = 38.05%), $n = 6$, ±S.E.M.

Table 2
Cumulative permeation of ketoprofen after 24 and 48 h

Ketoprofen	Gel I	Gel II	Gel III	Gel IV	Gel V
Q_{24} (μg cm^{-2})	633.48	1786.37	2000.76	3048.01	3425.55
Q_{48} (μg cm^{-2})	1500.20	2351.29	3071.11	4935.09	8108.61
Q_{24} (mmol cm^{-2})	2.49×10^{-3}	7.02×10^{-3}	7.87×10^{-3}	1.20×10^{-2}	1.35×10^{-2}
Q_{48} (mmol cm^{-2})	5.90×10^{-3}	9.25×10^{-3}	1.21×10^{-2}	1.94×10^{-2}	3.19×10^{-2}

The total amount of PG dosed in the case of t_0 gel was 0.894 mmol which, at approximately half the Q_{48} value indicates depletion occurred (as illustrated by the curved permeation profiles), and that this was attained rapidly. Moreover, the steady state permeation of ketoprofen observed with the t_0 gel (Fig. 1), which had the lowest concentration/thermodynamic activity, must have been a consequence of its simultaneous permeation along with the PG.

Squillante et al. (1998) studied the codiffusion of PG and dimethyl isosorbide across hairless mouse skin from infinite doses of formulations spiked with ^{14}C-PG. They observed zero-order permeation of PG, compared to non-zero order in the current work and a Q_{24} of 3 mmol cm^{-2}—an order of magnitude greater than the current work. These discrepancies can be attributed to the greater permeability of mouse skin compared to pig ear skin in conjunction with the application of infinite doses.

3.3. Simultaneous permeation of ketoprofen and propylene glycol

The molar ratio/time profiles of Fig. 3 shows that, not only did PG permeate skin simultaneously with ketoprofen, it was in excess at all times. The t_0 gel naturally demonstrated the largest excesses of ∼300 through ∼150 to ∼70 going from 12 to 48 h. This contrasts with other workers who found that steady state flux of PG began to diminish while permeation of the solute (loperamide) was still increasing (Trottet et al., 2004). This may be attributed to the application in the earlier work of a single finite dose of 10 or 40 mg cm^{-2}, coupled with the fact that 75% of their gel formulation was an unspecified co-solvent.

The double Y plots of Fig. 4 depict how the simultaneous permeation of ketoprofen and PG changes as a function of solvent content. If considered as representative of a drying formulation, it is clear that as solvent is lost from the formulation the nature of the applied film changes, with increased ketoprofen and decreased PG permeating the skin. Fig. 5 shows the correlation between the cumulative moles of ketoprofen and PG permeated for the test gels at 24 and 48 h timepoints. The decrease in the

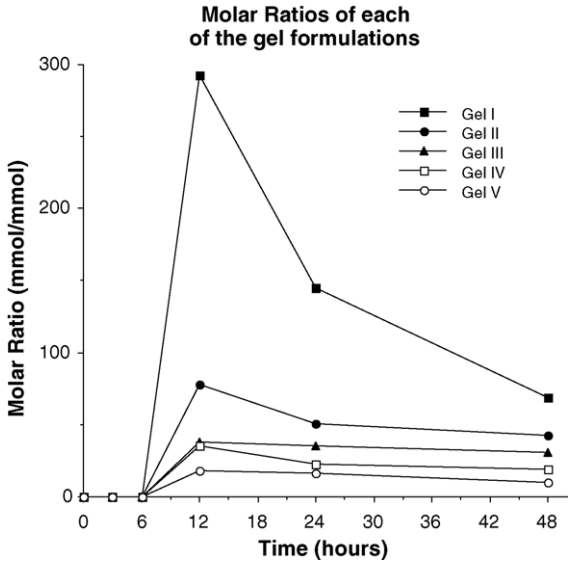

Fig. 3. Molar ratio, propylene glycol:ketoprofen permeated from Gels I to V as function of time from five gels with varying propylene glycol content (I = 90.91%, II = 88.48%, III = 84.68%, IV = 75.44%, V = 38.05%), ratio of two means, $n = 6$.

delivery of PG relative to the increase in delivery of ketoprofen was linear across the gel formulations.

It was reported that the dermal concentration profiles of pyrene butyric acid and propylene glycol into skin were similar via a solvent drag, or favoured-partitioning process (Schneider et al., 1996). They also found that mass ratios of the two species within the stratum corneum were fairly consistent over the study period of 1000 min. However, their work stopped short of determining the transdermal delivery of the two species, i.e. in the receptor phase. The permeation of PG was however, determined along with the solute, nifedipine, by Squillante et al., 1998, although the connection to the solvation of the solute was not made. Recent consideration of ratios of solute and polyunsaturated fatty acids in receptor phases gave rise to the notion that solutes can permeate skin complete with their solvation cages (Heard et al., 2003a,b; Karia et al., 2004). Fig. 3 suggests that

Table 3
Cumulative permeation of propylene glycol after 24 and 48 h

Propylene glycol	Gel I	Gel II	Gel III	Gel IV	Gel V
Q_{24} (μg cm^{-2})	2.75×10^4	2.31×10^4	2.14×10^4	2.09×10^4	1.49×10^4
Q_{48} (μg cm^{-2})	3.10×10^4	3.00×10^4	2.83×10^4	2.83×10^4	2.41×10^4
Q_{24} (mmol cm^{-2})	0.361	0.304	0.281	0.275	0.195
Q_{48} (mmol cm^{-2})	0.407	0.394	0.372	0.372	0.318

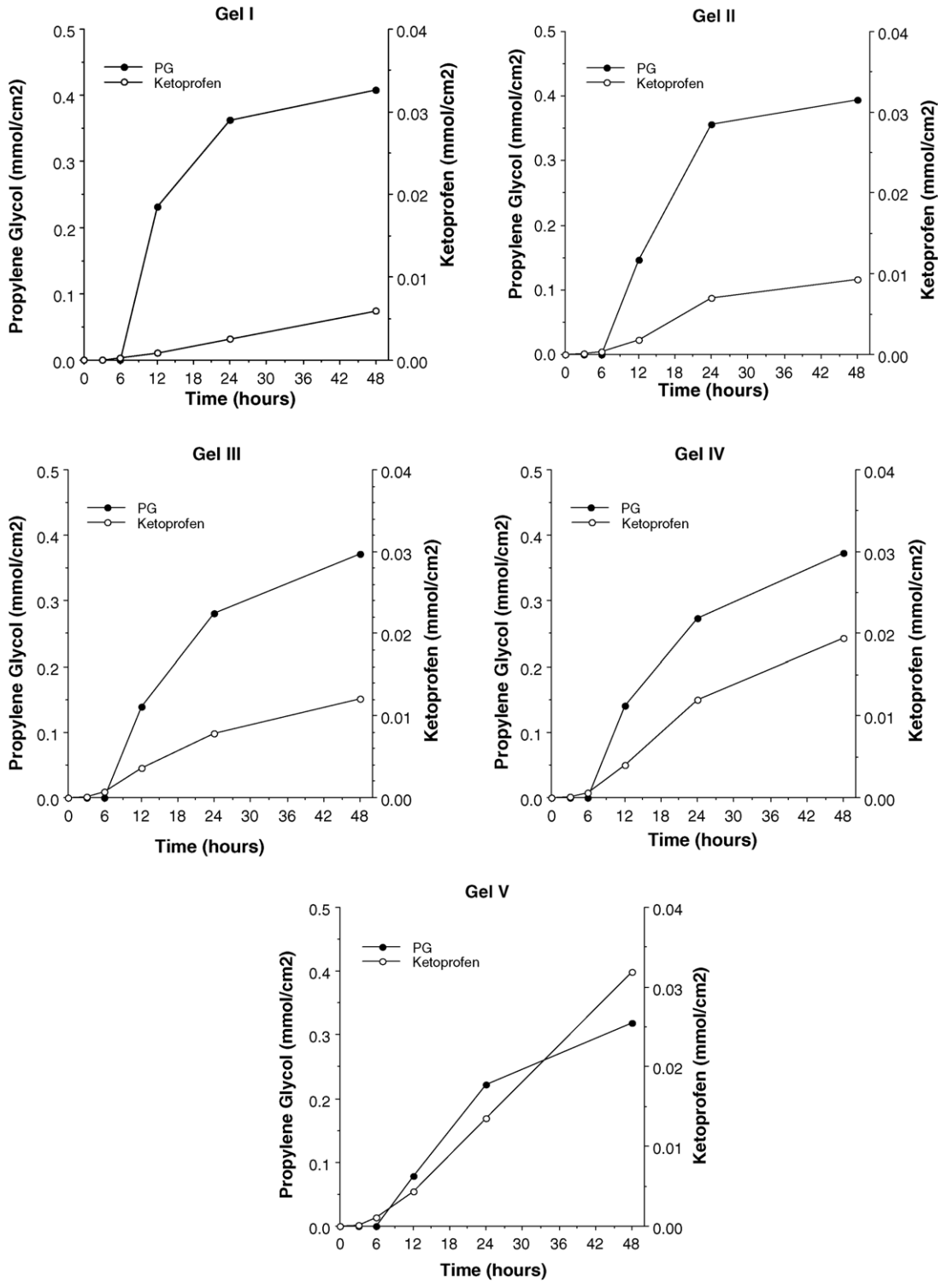

Fig. 4. Double Y plots showing the simultaneous permeation of ketoprofen and propylene glycol across pig ear skin over 48 h from five gels with varying PG content (Gel I highest, Gel V lowest), mean values shown (error as per Figs. 1 and 2).

as one approaches a state of dryness the ratio of permeated PG/ketoprofen stabilise across the duration of experiment, such as that for gel (V) the ratio is ∼12. Thus, it can be postulated that the solvation of 1 mol of ketoprofen involved ∼12 mol of PG—this is seemingly high, but may reflect the sum of tightly and weakly bound PG. Prior to this situation, a combination of solvated ketoprofen/PG complexes and free PG would have permeated the skin. It can be further hypothesised that the overall dimensions of the solvated complex would have an impact on the flux and k_p, thus the larger the complexant molecule the lower the rate of permeation—this is currently being examined in our laboratories.

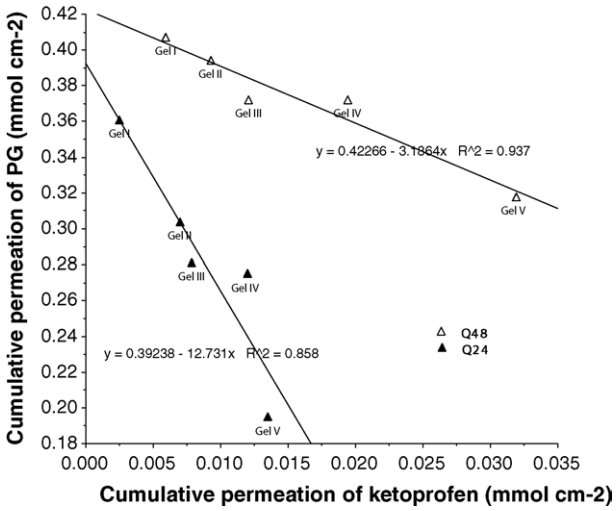

Fig. 5. Correlation between permeated PG (mmol cm^{-2}) and permeated ketoprofen (mmol cm^{-2}) at 24 and 48 h.

3.4. General discussion

The literature contains numerous works aimed at investigating skin penetration enhancers (Purdon et al., 2004) or enhancing excipients (Cornwell et al., 1998), although where one class ends and the other begins is indistinct. With a few notable exceptions, it is standard practice to implicitly conceptualise the permeation of active across skin in terms of individual molecules, devoid of any consideration of the fate of the solvent/excipient.

The earlier work involving the skin penetration of PG forms a useful backdrop to the current work. As early as 1985 PG was known to be a potent skin penetration enhancer and its ability to readily permeate skin noted (Hoelgaard and Møllgaard, 1985). In 1987 a 'pull effect' was proposed which enhanced skin permeation by promoting drug solubility in the skin-enhancer medium (Kadir et al., 1987), since referred to as a 'drag' effect, by other workers (Bendas et al., 1995; Sloan et al., 1998). Such terms are somewhat misleading as they infer that the solvent permeates and modulates the skin prior to commencement of permeation of the solute. Regular skin permeation theory tells that a permeant molecule will diffuse in the opposite direction to its chemical potential. However, if it is thermodynamically less favourable for a solute to dissociate from its solvated molecule of vehicle than to remain associated (Perlovich et al., 2003) then the solvated complex must partition into the skin and permeate across it rather than a discrete, de-solvated solute molecule. If the solvent also permeates the skin then the solute contained within it as a dispersion of solvated complexes will also permeate, given a homogeneous solution, from the time of application. The work of Trottet et al. (2004), where time-dependent flux maxima which were different for loperamide and PG were found, may appear not to support this hypothesis, but their formulations contained 75% unspecified solvent which probably also interacted with or solvated the loperamide.

A principal element in Fickian diffusion is a 'step' involving the partitioning of active from formulation into skin and log *P* has become a fundamental consideration in skin permeability prediction. In practice, log *P* is not always a reliable predictor, for example, Bendas et al. (1995) noted a lack of correlation between penetration and partitioning data involving steroids in PG/water mixtures. If a formulation or elements thereof are also partitioning into the skin as well as the solute drug, then the issue of partitioning is of questionable relevance, at least in isolation from the partitioning of the solvent. Furthermore, any reservoir formed within the skin under such conditions will be one that is comprised of the solute and the solvent (Younick et al., 2004) which also implies that the thermodynamic activity of the system is also of questionable relevance. This would help explain some of the data that do not conform with thermodynamic activity theory as related to skin permeation. Significant uptake of solvent and solute would also explain the levels of permeants found in skin that were much greater than the amount of extractable lipid previously observed (Heard et al., 2003a,b; Banning and Heard, 2002). Another factor relates to the physical process of application, where massaging the formulation substantially enhances the initial drug/solvent absorption event relative to a non-massaged dose (unpublished data).

The enhancement mechanism of PG is often stated in terms of its interactions with skin lipids (Williams and Barry, 2004)—it is often overlooked that at levels of >40% it is keratolytic (Page et al., 2002) and that it can produce conformational changes of keratinised protein from α-helix to β-sheet (Takeuchi et al., 1992). This may be related to the observation made by Bendas et al. (1995) that for hydrocortisone butyrate the drag effect became operational at a level of PG 60+%, and below 40%, permeation was thermodynamically controlled. In the current work, we were aware that the use of large proportions of PG may thus have compromised the skin barrier, but the observed steady state fluxes of ketoprofen suggested this was not the case. The same data show that the massaging action used to dose the cells did not have any deleterious erosional effects on the stratum corneum.

In the current work there did not appear to be any excessive binding of ketoprofen to the hydroxypropyl cellulose thickener, unlike in previous studies which reported a considerable interaction between the active and Cabosil M5 was noted M5 (Gallagher et al., 2003a) which had significant ramifications on the amount of drug delivered into and across skin (Gallagher and Heard, 2005). The choice of thickener is therefore a critical in determining the retentive potential of the formulation.

4. Conclusions

Using a massaged multiple finite dose protocol (representative of in-use application of topical formulations) it was found that as a formulation dries post application the film will change in a way dependent upon a number of factors including the binding potential of the excipients and the proportion of absorbable solvent. The snapshot approach to modelling solvent changes in topical formulations allows one to probe time points in the topical delivery event in isolation. Quite clearly excipients and solvents used in topical formulations can permeate skin and potentially be absorbed into the system. PG, a potent skin penetration enhancer, probably represents a worse case scenario,

although the drag effect is potentially a major and understated means by which actives are delivered across skin. However, the concept needs to be considered in terms of the solvation characteristics of the solute within the medium and the permeation of complexed species across the skin. Although perhaps arduous, it is important to account for the penetration/permeation of all species within a formulation to fully rationalise the delivery of the main active/solute.

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